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SOME CONSIDERATIONS REGARDING SO-CALLED FORMATIVE SUBSTANCES.

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Within the last few years the idea of "formative substances" has received consideration and support from workers in certain fields of zoölogy and embryology to such an extent that it appears worth while to examine critically some of the assumptions upon which the hypothesis of the existence of such substances rests.

As the first step in this consideration a definition of formative substances is necessary. But anything like an exact definition is difficult to find even in the literature which has devoted most attention to these hypothetical substances. We read of ectodermal substance, myogenic substance, hydranth-forming substance, head- and tail-forming substances, etc., but the general question, what is a formative substance? does not seem to be very clearly answered. It may, however, be inferred from the use of the term that a formative substance is a specific substance capable of giving rise under certain conditions to a specific structure. Secondly, we must inquire as to the nature of organic structure to which these formative substances are supposed to give rise? Organic structure may be defined as a complex of heterogeneous parts of typical constitution and arranged in typical space relations. Strictly speaking this definition is not exact, for organic structure, like other kinds of structure, is fundamentally simply a matter of space-relations or arrangement of parts entirely independent of their constitution. The constitution of the component parts must of course play a rôle in determining the dynamic or functional activities of the structural complex, and ordinarily we do not

clearly dissociate the idea of organic structure from the dynamic activities which it exhibits. For the sake of critical analysis, however, it is necessary to emphasize the fact that organic structure is primarily a typical arrangement of heterogeneous components without regard to their constitution. This becomes evident at once from the following consideration: if each component of a given organic structural complex could be distinguished it would be possible to build up artificially an identical structure in which each component was different in constitution from the corresponding component in the original structure. On the other hand, if all the different components of an organic structural complex could be isolated without altering their condition and then mixed together the mixture would not represent the original structure.

It thus becomes evident that the space-relations of the components, not their constitution, are the characteristic feature of structure proper. From this conclusion it follows that formative substances, if they exist, must be specific substances which determine the arrangement of the component parts of a structural complex. In order to determine whether formative substances do exist we have first to consider the possible methods of origin and action of such substances. As regards the individual, for example, each substance must either be represented in the primitive germ-cell by an ultimate "unit of organization" of the same nature as itself, or it must have arisen from other substances in the course of development.

In the one case development is merely the "unfolding," "activation," or whatever we prefer to call it, of preformed "germs," "determinants," or "definite, determinate and primary" units of organization. In the other case it is, properly speaking, an epigenesis, in the course of which new formative substances appear as the result of the activity of others, and the complexity of the developing organs increases.

Directing our attention first to the preformation hypothesis we must inquire as to the nature of these ultimate units which represent both the formative substances and the structures of later stages. Are they simply molecules or groups of similar molecules, or are they something more complex?

Ultimate Units Molecules or Groups of Similar Molecules.

If the ultimate units are molecules or groups of like molecules, then their properties as formative substances must be the result of their physical and chemical properties as molecules. If this be admitted the hypothesis based on these assumptions is identical with or similar to the crystallization hypothesis. For structure is primarily a definite and characteristic space-relation of parts and an aggregation of like molecules cannot, so far as we know, give rise to anything like definite and characteristic fixed space-relations except by an arrangement of its molecules according to physical laws. According to this view organic structure is made up of a multitude of component parts, each consisting of like molecules, but each different from the others in composition and each arising from its corresponding ultimate unit. But in the fully developed structure these different components are definitely arranged, and indeed it is this feature — the definite space-relations of heterogeneous parts — that is the fundamental characteristic of all structure. To what is this arrangement due? If we say it is due to the physico-chemical interactions and relations to each other of the substances in a given environment, then we have departed from our premises, for in this case the relations and the environment are more directly and immediately formative factors than the substances. The substances have formed the various components, but there is no substance which can be made to account for their arrangement.

If, on the other hand, we assume that the characteristic arrangement of the heterogeneous parts is due to something else than their physico-chemical interactions and relations in a given environment, then we pass beyond the realm of physics and chemistry and become "vitalists."

In short if we start from a series of molecules or groups of like molecules as the ultimate formative substances, we can, by assuming a process of crystallization or something similar in each substance, account for the development of each physico-chemically homogeneous element of structure, but we cannot account for the most important feature of structure, *viz.*, the characteristic arrangement of heterogeneous elements except by assuming rela-

tions and interactions as the formative factors or by adopting a vitalistic point of view. In any case the formative substances are inadequate to produce the observed result.

But the crystallization hypothesis has found no wide acceptance. Most authorities agree that the process of formation of the various parts of organic structure is at least in most cases either only very remotely similar to the process of crystallization or differs totally from it. It may be that in certain cases the process of morphogenesis is akin to crystallization but the results of experimental work demonstrate that in very many cases it certainly is not.

Ultimate Units Groups of Dissimilar Molecules.

The other alternative under the preformation hypothesis, *viz.*, that which assumes the ultimate units or formative substances to be something more than molecules or groups of like molecules, also presents certain difficulties.

These complexes must be either simply physical mixtures or complexes or they must be molecular complexes of some other kind.

If we consider them as physical complexes then their formative activity is the result of the relations between parts or of these relations plus a given environment, *i. e.*, none of the substances can be regarded as in itself formative but only the complex as a whole or the complex plus the environment.

Now if the formative substance is a mixture or other physical complex whose activity depends only or primarily on relations between its parts why does its activity appear only at a certain time? If we admit that the environment, *i. e.*, relations with other elements, etc., play a determining rôle then these relations are just as truly formative as the complex on which they act. Moreover, as in the case considered under the previous heading we must either assume that the characteristic arrangement of the elements in developed structure is due to physico-chemical relations between the different complexes or we must take refuge in vitalism. But let us assume that the ultimate element is a complex of molecules of some other sort, *i. e.*, that it possesses a certain "organization." Physics and chemistry recognize at

present no such organization of dissimilar molecules. Thus we are again in danger of being regarded as vitalists. Even if we assert that this organization is finally reducible to a physico-chemical basis we cannot show how it is reducible. In any case we are adopting a position for which the facts afford no warrant; moreover, we are assuming in our hypothesis exactly that which the hypothesis is supposed to explain, *i. e.*, we postulate one organization in order to explain another. Naturally if organization forms the basis of the hypothesis no real difficulties will be encountered in individual cases for we can refer them at once to the hypothetical organization. One frequently reads that a considerable degree of organization is necessary for the phenomena of life, that protoplasm possesses a high degree of morphological organization, etc. The authors of these statements do not always inform us as to the nature of this organization, but there is, I think, no escape from the conclusion that unless we admit that a molecule or a physical complex of molecules can exhibit the phenomena of organic life our hypotheses are open to the objections stated above.

In his earlier work Weismann designated the ultimate units postulated in his hypothesis as molecules but later asserted that they are not molecules in the physical sense, but consist of a number of such molecules. Unless these complexes are purely physical, and so far as I can determine, Weismann does not seem to regard them as such, his hypotheses is open to these same objections. Other similar "organization" hypotheses are in the same position.

The Hypothesis of Epigenetic Origin of Formative Substances.

If a development of new formative substances from previously existing substances occurs during development we come ultimately as regards ontogeny to the primitive germ-cell which must be composed either of a formative substance or of a complex of substances from which the substances appearing in later stages arise. At the present time probably no one would regard the primitive germ-cell as containing only a single formative substance but such a hypothesis would be a contradiction in terms

since the real formative activity would in this case reside in the environmental conditions not in the substance.

Assuming then that the primitive germ-cell contains a complex of formative substances all that has been said in the discussion of preformation hypothesis regarding the nature of ultimate units of organization would apply here. The complex must be physico-chemical in nature and the formative factors must reside in the relations between the constituents of the complex and in the environment, not in the single constituents themselves, for a single substance cannot of itself give rise to unlike substances, definitely arranged.

Most hypotheses of this character assume further that the elements of this ultimate complex are definitely and characteristically arranged. This arrangement must be the result of physico-chemical conditions past or present, *i. e.*, the cause of the organization is to be found in relations not in particular substances, or else we must again take refuge in vitalism and assume the existence of some organizing principle ultra-physico-chemical in nature.

The next question to be considered is — how do the new formative substances which appear at any given stage and in typical space relations arise from those previously existing? Here again we must point to the physico-chemical conditions in the complex, *i. e.*, to the relations and interactions of the elements in a given environment as the real formative factors, since a single substance cannot of itself give rise to unlike substances in typical space-relations.

And finally, how do the last series of formative substances give rise to the definitive structures? Here three points of view are possible. We may assume as in the preformation hypothesis a process of crystallization or something similar but by so doing we account only for the form of the elements of structure not for the characteristic arrangement of these elements. We may attempt to account for this by assuming that the formative substances existed in a similar characteristic arrangement, but this only throws the difficulty back to earlier stages. We must turn sooner or later from substances to relations or else to vitalistic assumptions. Secondly we may assume some grouping principle

of unknown nature — again a vitalistic conception — or finally we must admit that the relations not the substances give rise to the final arrangement of parts.

THE RÔLE OF CHEMICAL CONSTITUTION IN MORPHOGENESIS.

Objection may be made to the conclusions reached above on the ground that the chemical constitution of the various substances plays a part in determining the character of the relations which exist between them, and consequently in determining the character of the result. It is of course quite true that the chemical constitution of the substances involved may affect the result, but a single substance can accomplish nothing of itself by virtue of its composition. It must enter into relation with another or others in a more or less typical environment and the composition of the other substances and the factors of the environment are equally important in determining the result. What are the real formative factors in this case? It is evident that we can properly recognize only a formative complex of substances and relations and in this complex the constitution of the various substances is a condition affecting the character of the result while the relations between the substances are the cause of the result. But again it may be objected that the chemical constitution is the cause of the relations existing. While this may be true in large measure for certain simple chemical reactions it certainly is not true for many of the processes which give rise to organic structure.

Many other conditions beside those resulting from chemical constitution are usually important. For example, we know that the formation and persistence of various structures is determined primarily by simple mechanical conditions. It may be said that these conditions are not effective unless they act upon a particular substance but unless we make the term particular substance equivalent to protoplasm of a particular species or genus there is no evidence that this is true.

There is very strong evidence for believing that mechanical conditions resulting from the presence of fluid in a cavity are often fully as important as the character of the cells about it in determining the development of hollow, fluid-containing structures. We know that mechanical conditions are potent factors in the de-

velopment of bone and connective tissue and many other similar cases might be mentioned. It is conceivable that in some formative processes the most important conditions are chemical relations, but even in such cases the chemical constitution of a given substance is only indirectly "formative."

Moreover, in many other dynamic phenomena occurring both within and outside of the organism the chemical composition is of little importance. The catalytic activity of colloid solutions of metals does not appear to differ fundamentally from that of enzymes and yet the substances involved are widely different; in many of the effects produced by electrolytes one chemical element or radical may be substituted for another without altering the result: compounds of widely different chemical composition may produce identical osmotic phenomena, etc. In short, chemical constitution is at best only one of a large number of factors involved and often is of little or no importance.

Morphogenetic properties have been assigned by various authors to specific enzymes and to other substances of more or less definite chemical constitution. For example, in a recent paper[1] L. Loeb makes the following statement: "Ferments produce primarily chemical changes. But we know of chemical ferment actions which bring about structural changes in the medium in which they act. Thrombin in transforming fibrinogen into fibrin changes a colloidal fluid into a gelatinous, more or less solid mass, which under the influence of pressure and traction may show a fibrillar structure not unlike connective tissue. From a certain point of view the fibrin ferment may therefore be regarded as a form-producing ferment. We might call it a morphogenetic ferment. We have reason to assume that there exist other morphogenetic ferments" (p. 150).

A critical examination of this statement will at once render it evident that the real formative factors in the production of the fibrillar structure are the pressure and traction, and not the enzyme. The enzyme simply changes the condition of the medium in which it acts in such manner that pressure and traction produce a visible structural effect. But the enzyme itself is not properly speaking morphogenetic. The other cases

[1] Immunity and Adaptation. BIOL. BULL., Vol. IX., No. 2, 1905.

of so-called morphogenetic enzymes seem to be similar to this one in that the structure appears, not as the result of enzyme action, but in consequence of certain physical conditions which may accompany or follow the enzyme action but are not an essential part of it. There is nothing specific in the relation of this process to the enzyme. Coagulating or coagulated colloids may be made to assume a fibrillar structure in many cases "under the influence of pressure and traction" in total absence of enzymes. The specific effect of the thrombin and of other enzymes as well has not in any case been shown to be morphogenetic in character.

In general, physical conditions appear to be more important factors in morphogenesis than substances of particular chemical constitution.

Formative Substances in Ontogeny.

The chief reason for the consideration recently accorded to this old idea of formative substances appears to lie in the fact that many eggs exhibit visibly differentiated regions which normally give rise to particular structural complexes. Moreover, experiment has demonstrated that in some cases certain of these regions are capable of continuing the process of visible differentiation in a manner apparently normal after separation from other parts of the egg or embryo. The conclusion in such cases is that these regions must contain a certain substance or certain substances which are responsible for the differentiation. Hence these hypothetical substances are called formative. These regions are mostly extensive and give rise in development to a multitude of structures : for example we read of ectodermal formative substances, neurogenic substances, myogenic substances, entodermal substances, etc. In some cases, however, smaller regions giving rise to definite organs or parts of organs appear to possess in greater or less degree the power of "self-differentiation." We must regard these regions from either the preformation or the epigenetic point of view, *i. e.*, each element of the structure to which they finally give rise must be represented by an element existing before visible differentiation or else new elements must arise in the course of development. In any case the region must

be a complex. All that has been said above regarding complexes of substances applies here. The result produced is the result of the sum total of conditions existing in the complex. When we assume that the result is due to the presence of certain specific substances in the complex which manifest themselves in a peculiar "formative" fashion we are not only making an inference not warranted by the facts, but we are involving ourselves in various difficulties which become manifest only on careful analysis. Some of these I have endeavored to point out above. All the substances in the complex are or may be formative, and under certain conditions the region gives rise to a characteristic differentiation. But if all the substances are formative the distinction of formative substances is entirely unnecessary and we must regard the region merely as a formative complex.

But it may perhaps be maintained that the formative substance hypotheses do not differ essentially from this since the formative substances are really complexes, not definite chemical substances. If this is the case then it is certainly preferable to avoid the use of the vague term substance in this connection, especially since "formative" factors are known in many cases to be related to substances only very indirectly. Moreover, it is difficult to understand the grounds for distinguishing a particular complex either of substances or of conditions as formative since this implies that others are not formative. The only conclusion justified with regard to "self-differentiation" in isolated blastomeres or egg-regions is that the conditions for the observed differentiation reside in the part. This, however, is really quite different from the conclusion that the piece contains certain formative substances for the particular structures to which it gives rise. The region may differ chemically in greater or less degree from other regions of the egg and this difference may be more or less closely related to the structural result and to that extent formative. But to dignify the chemical peculiarity of a region by the term formative substance involves unwarranted assumptions. The strongest evidence in support of formative substance hypotheses is found in these cases of ontogenic self-differentiation of parts and this, as has been seen, is far from conclusive.

In order to avoid misconception it should perhaps be stated

positively that the writer's position does not at all involve a denial of the clearly demonstrated fact that these regions are different or have become different from others in the egg or embryo, nor does he underestimate the value of the observations which have directed attention to this fact. But to say that these regions contain specific formative substances is to say too much, for the visible differences do not necessarily stand in direct relation to their formative activity. The same "kind" of protoplasm is certainly capable of widely diverse formative reactions under different conditions and the determining conditions are demonstrated in many cases to be dynamic rather than substantial. It seems more nearly correct therefore to maintain that particular lines of activity have been initiated in these formative regions in consequence of past or present conditions and may continue even after isolation of the regions. That different substances exist in the egg and embryo is also clearly demonstrated, but to assert that these are formative or that their presence indicates the existence of particular formative substances is quite another matter, and as the writer believes, not justified by the facts. Should we not therefore be content in view of the facts to designate these so-called "formative" or "morphoplasmic substances" merely as cytoplasmic differentiations, leaving the question as to the nature and significance of the differentiation open?

Formative Substances in Regulation.

The attempt has recently been made by Morgan[1] to account for certain phenomena of regulation, especially those connected with polarity by postulating the existence of formative substances. This hypothesis is selected for discussion since it is the latest attempt to interpret formative processes in this manner. Other similar hypotheses are, however, open to most of the same objections. In addition to the objections discussed above to which the idea of formative substances in general is open there are certain other objections which apply specially to the application of this idea to the phenomena of form-regulation.

It is of interest to note first that these formative substances are quite different from those of the embryologists. Here for exam-

[1] *Science*, XX., December, 1904. *Journ. Exp. Zoöl.*, I., 1904, and II., 1905.

ple instead of ectodermal, neurogenic, myogenic, mesenchymal, entodermal, substances, etc., we have head-forming and tail-forming substances, pharyngeal substances, hydranth-forming material, stolon-forming material, etc. How do these substances arise from the substances supposed to exist in ontogeny. A head or tail of a planarian or the hydranth of a hydroid is a very complex structure which has developed from regions more or less widely separated in the egg. Is the head-forming substance made up of all the different substances which were concerned in the original development of the head or is it something new? If it is the sum total of the ontogenetic substances then it must in itself be organized, *i. e.*, the different parts of which it consists must possess characteristic positions with reference to each other and we have another organization to account for. Moreover, why should such an enormous amount of head- and tail-forming substances as must exist for example in planarians be left over after development is completed? What is their function in the normal animal or are they without function? If the latter is the case it looks very much as if provision were made in the normal animal for regulation, in other words as if regulation might be after all an adaptation.

Polarity is regarded as identical with a postulated gradation of the various materials. The head-forming materials in planarians, for example, are supposed to decrease from the anterior end backward, the tail-forming materials in the opposite direction, the pharynx-forming material from the middle toward both ends, etc. This idea followed to its logical conclusion certainly does not assist us in comprehending organic structure, but rather increases the difficulty. How did this gradation come about and why should it exist? As we have seen, the various head-forming substances were widely separated in the egg. How and why have they combined in this peculiar manner? Why does not a small piece from the region of the body just behind the head in *Planaria* always give rise to heads at both ends, since the head-forming substance is greatly in excess at both ends as compared with the tail-forming substance? Why does not the pharynx in such a piece arise at the extreme posterior end since the pharynx-forming substance is more abundant there than elsewhere in the piece?

In considering the case of *Tubularia* Morgan[1] makes the following statement which may perhaps serve to illustrate the nature of his hypothesis: "We may assume that the gradation of the material is of such a kind that the hydranth-forming material decreases from the apical toward the aboral end. The formative influence acting from the exposed end inward (the stimulus of the water on the free end), finds a prompter response when it acts in the direction of decreasing amounts of hydranth-forming material (which has the same gradation as that in the hydranth itself) than when acting in the reverse direction (namely at the aboral end). Therefore the oral polyp, as a rule, develops first. For its development it needs certain nutritive material. This it finds either in the cœnosarc or in the circulation, and uses the materials as it develops. In consequence the cut surface at the basal end cannot get the material necessary for it to develop into a hydranth and it either remains undeveloped or produces a stolon."

Besides the assumption of the gradation of material this statement of the hypothesis contains a number of other assumptions. The rapidity of response to the stimulus depends on the direction of gradation of material; a gradation of hydranth-forming substance exists in the hydranth itself. The hydranth takes up nutritive material and so prevents the aboral end from obtaining it, so that this produces a stolon or nothing. But why should the rapidity of response depend on the direction of gradation? What reason is there for supposing a gradation of hydranth-forming substance in the hydranth itself? Why should a stolon develop because the aboral end cannot obtain the nutriment which is taken by the developing hydranth? Does the developing stolon require no nutritive material or does it require a different kind from the hydranth? If the latter, then stolon-formation and logically also hydranth-formation are due not to stolon-forming and hydranth-forming substances laid down in the stems in regular gradation but to nutritive substances occurring apparently anywhere. Moreover, we are told that the formative influence is the stimulus of the water on the free end. If this is the "formative influence" what need is there for hydranth-forming substance?

[1] *Journ. Exp. Zoöl.*, I., 4, 1904, pp. 587–588.

I find it very difficult to obtain any definite conception of the character of the hydranth-forming substance or of the manner in which it acts. Apparently it is not responsible for the size of the structure but only for its characteristic form. In the further development and discussion of the hypothesis a large number of special assumptions are made to meet special cases.

Loeb has recently asserted his belief in the existence of formative substances in connection with regulation and especially as determining polarity. Loeb's view appears to resemble the older Bonnet-Sachs hypothesis of the migrations of particular substances in particular directions. According to Loeb the formative substances are nutritive in character. This hypothesis does not appear to meet the objections to which other hypotheses are open and introduces some new difficulties since conditions determining the localization and distribution of the nutritive material do not seem to be considered.

On the other hand, various results have been obtained in work along this line which hypotheses of formative substances do not seem to me to account for. To mention only a few of these, in *Leptoplana* the larger the portion cut off posteriorly the larger the amount of new tissue formed at the posterior end of the piece remaining. Thus a piece comprising only the anterior third or fourth of the body will produce without being fed five or six times as much new tissue as the anterior four fifths. Yet the "tail-forming substances" and nutritive substances must be much more abundant at the posterior end of the longer piece than at that of the shorter. Again, of two pieces of *Leptoplana* with posterior ends at the same level one containing the cephalic ganglia produces a larger tail than one without them. Why should this difference occur, since the tail-forming substances are the same in amount at the level of the cut and the amount of nutritive substances must be approximately the same in both cases. In *Cestoplana* the new pharynx appears at a given level in a piece containing the ganglia and farther anteriorly in a piece from the same region without the ganglia.

Objection may be made to the citation of these cases here on the ground that they are not pertinent to the matter in hand, in that they are not connected with the problem of polarity. It seems

to me, however, that the relative size of structures produced from different levels and the relative position of intermediate structures are as truly an expression of polarity as the existence of different structures at the two ends, since they must be the result of characteristically different physiological conditions present at the different levels.

Though I have not attempted it, it is possible that with the aid of special assumptions these cases could be made to fit into a formative substance hypothesis, but the value of a hypothesis is to a certain extent inversely proportional to the number of special assumptions required, and there is always danger that a special assumption may introduce more difficulties than it removes.

Moreover, if axial polarity is due to a gradation of substances, we must logically conclude that a gradation of substances from the median line to the lateral margins of the body in both directions exists, for the structural differentiation in these directions is just as truly an expression of "polarity" as is the axial differentiation. This leads us into numerous further difficulties. This lateral gradation must exist in the developing parts as well as in the old, otherwise the differentiation between lateral and median regions could not arise. How, for example, are we to account for the formation of the other side of the body and of a median region in longitudinal pieces of planarians of less than half the width of the body? In whatever manner we may proceed to bring these cases under the general hypothesis certain other special assumptions are necessary. If we say that the formation of the opposite side along the cut surface is due to the excess of margin-forming substance in the piece, how shall we account for the formation of a median region between the two margins? If, on other hand, we attribute the formation of a median region to gradation of the formative substances for this region, supposing them to be in excess at the cut surface, how shall we account for the formation of the opposite side? Furthermore the new marginal region is formed first. Consideration of other experimental data adds further difficulties.

But perhaps enough has been said to show some of the special difficulties in which a hypothesis of formative substances involves

us when we attempt to apply it to the phenomena of form-regulation. Some of the special cases discussed by Morgan will be considered more fully elsewhere in connection with new experimental data.

Such a hypothesis appears to me a totally unnecessary assumption in most cases, probably in all. Moreover, it does not afford a satisfactory explanation of the facts. I have recently made an attempt to account for some of the facts of regulation in a somewhat different manner,[1] and without assuming the existence of a series of substances which seem to me to make comprehension more difficult.

The Nature of Formative Processes.

The chief peculiarity, and, as I believe, the fundamental error in the hypotheses of formative substances seems to be that they regard the process of morphogenesis as something *sui generis* and not as simply a part of the dynamic or functional activity of the organism. The activities of the organism are considered as two-fold, one group being concerned with the construction of a complex machine, the other with the functions of that machine when completed. According to this view development is primarily a period of construction and not of function. This idea is perhaps a natural consequence of the separation of morphology from physiology. But when we consider the data already at hand it seems impossible to make any such distinction. The organism is primarily and at all times a dynamic or functional complex and the process of morphogenesis is merely an incident, or, in other words, structure is a visible by-product of these activities. In short, it is rather the result of the relations of parts than of any "formative" capacity existing in the single elements themselves, and as regards any given element or unit of the organism the factors of the environment and not the element itself must be, as we have seen, in the final analysis, the real formative factors. These environmental factors may be otherwise designated as the functional or dynamic conditions and we may regard development as primarily a functional process.

[1] *Roux's Archiv.*, XX., 3, 1906.

It is, of course, quite true that the character of the dynamic or functional conditions at any given stage is determined in greater or less degree by the character of the structural consequences of previous activities. These new activities give rise to new structural results until a certain stage is attained, determined doubtless by the complexity of the physico-chemical constitution of the organism, where the mutual determination of structure and dynamic conditions maintains a more or less exact equilibrium for a time. At this stage development is said to be completed. Any or all of the dynamic conditions may in proper environment give rise to conditions visible as structures, hence there are no good grounds for distinguishing certain groups of them or of the substances or structural complexes in connection with which they arise as formative from other merely "functional" groups.

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THE FORMATION OF NEW COLONIES OF THE ROTIFER, MEGALOTROCHA ALBO-FLAVICANS, EHR.

FRANK M. SURFACE.

Colony formation is not common among the Rotifera. But in several species, belonging especially to the family Melicertidæ, the individuals do become aggregated into colonies. These animals do not reproduce by budding, as do so many colonial forms, nor in most cases does the colony contain the progeny of its members. Instead, the young are hatched as free swimming individuals which later become segregated into a separate fixed colony.

During the past winter, while a graduate student at the University of Pennsylvania, the writer made some observations on the formation of new colonies of *Megalotrocha alboflavicans*. Colonies of this large rotifer were found in abundance in the fresh-water tanks of the vivarium, whither they had been transferred some years before from the small pond in the botanical gardens. These colonies are ordinarily formed on the roots and stems of various water plants, but in the vivarium they were found only on the stems of *Myriophyllum*. The colonies are nearly spherical in shape and when adult may measure as much as 4 mm. in diameter. They are thus easily recognizable to the unaided eye, appearing as white spots on the dark or green stems. The colonies used were kept in glass jars in the laboratory and for observation a piece of the stem containing a colony was removed, placed in a small dish and observed with a Braus-Drüner binocular.

The work was undertaken at the suggestion of Professor E. G. Conklin, and it is a great pleasure to acknowledge my indebtedness to both him and Professor H. S. Jennings for their many valuable suggestions and kindly criticisms.

The colonies of *Megalotrocha* are not surrounded by gelatinous masses or tubes as are certain other species of the Melicertidæ.

The animals are however attached to the stem by a kind of mucilaginous substance secreted by a gland in the foot. This substance forms a thin layer on the stem over the area of attachment of the colony. The adult colonies usually contained eggs or at least soon produced them after being brought into the laboratory. The eggs are attached to the mother by means of an adhesive gland situated a short distance posterior to the opening of the cloaca. Usually only one or two eggs are found attached to a single individual, but sometimes as many as four were observed.

The method of egg deposition is interesting. The egg rapidly increases in size in the region of the vitellarium, and then passes slowly down the oviduct to the cloaca. When the egg with its large germinal vesicle has reached this region the animal bends towards the dorsal side in such a manner that the adhesive gland touches the protruding egg. The animal frequently remains in this position for some time, often bending still farther so that the corona points towards the foot. In this way the end of the egg is firmly pressed against the adhesive surface of the gland. The animal now slowly and by repeated attempts straightens itself and at the same time the egg is pulled from the cloaca and remains attached to the mother, where it undergoes development. One egg has scarcely been deposited before another can be seen enlarging in the region of the vitellarium. Eggs begin to form in all the individuals of a colony at approximately the same time. But an interval of three or four hours may elapse from the time the first egg is laid until all the animals have deposited eggs. During this time the individuals which deposited the first eggs have often deposited a second. This overlapping of broods is important, as will be pointed out later, in keeping the size of the colonies more nearly constant.

The length of time required for the young rotifers to hatch varies somewhat, depending on the temperature and other conditions. Usually they hatch in three or four days after deposition. The young rotifer when fully formed can be readily seen through the transparent egg membranes. For some hours before hatching frequent contractions of the body and movements of the cilia and mastax may be seen. By means of these contractions the

young animal finally bursts the enveloping membrane and is able to swim about. The young rotifers possess an organization in most respects similar to the adult. But among the more important differences may be mentioned the following. The trochal disc is at this time no broader than the trunk, the whole animal tapering slightly towards the foot. At the foot there is a circlet of small cilia. On the anterior border of the trochal disc, near the dorsal side are two red eye spots which are lacking in the adult. The cement gland in the foot is proportionately larger in the young animal than in the adult.

The young rotifers are free swimming, but are always attached at the posterior end by a thread of adhesive material which they spin out much after the fashion of a spider. They swim about among the adult rotifers, sometimes venturing a short distance beyond the limits of the colony but always drawing back and continuing to move about among the old animals. During all this time they are attached to the old colony by the adhesive thread from the foot. By a continuation of this nervous crawling and swimming some of them finally get their webs so twisted together that they are brought into contact. Here they remain, apparently without secreting more of the adhesive thread. At first there are only two or three individuals thus approximated and these are near the center of the old colony. Soon other young rotifers get their webs entangled with that of the few aggregated ones and these are then added to the ball that is forming. Here as well as later, contact with the adhesive thread appears to act as a guiding stimulus to the young rotifers. As soon as a young animal comes in contact with the rather thick thread leading to the forming ball, it at once begins to move up or down this thread until it finally reaches the ball; this I have repeatedly seen. Usually the young animal does not at once attach itself to the ball, but continues to crawl about, often through the bunch of young rotifers. In this way it finally becomes attached to its comrades with its foot closely adhering to theirs. During all the time that the ball is forming, the individuals composing it act in a very nervous manner, constantly jerking, twisting, contracting and expanding in a most irregular way. By means of this continual twisting and squirming the forming ball succeeds

in stretching the thread holding it fast, so that later it comes to lie outside the limits of the old colony (Fig. 1). In the course of time this thread breaks and the young ball swims away.

From the time the first individuals are hatched until the ball breaks loose there is usually a lapse of three or four hours. Since all the eggs of one brood are not deposited at the same time the period of hatching often extends over several hours. The long time that it takes for the ball to form and break away gives opportunity for the later individuals to hatch and get into the new colony. Often there are a few young that do not get

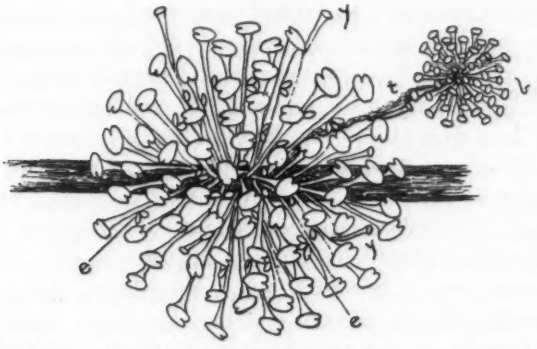

FIG. 1. Sketch to show formation of the swimming ball. *e*, eggs, attached to the adults; *y*, young animals before coming into the ball (*b*); *t*, mucilaginous thread holding the ball to the mother colony.

into the ball before it breaks away. These swim about for a time near the old colony and then may settle down on a near-by stem. Apparently these do not long survive the attacks of their enemies, for later I have seldom been able to find them, and I have never seen an isolated adult. By thus losing a few individuals in every generation the colonies would evidently continue to get smaller, for after the ball breaks away no new individuals are added to the colony. Such a decrease in numbers would undoubtedly take place were it not for the overlapping of the broods. One generation of eggs is laid so soon after the other that, in many cases, a large number of the second brood hatch in time to get into the forming ball. Occasionally, but not often,

two balls are formed at the same time, thus dividing the young colony.

The ball of young individuals is spherical in shape and is very regularly formed. Indeed, this is one of the very striking things about these interesting balls. The feet of the individuals are not attached together in an irregular mass, as one might suppose, but they are arranged so as to form a very regular sphere. It is difficult to ascertain just how the animals are held together in these balls, but it seems probable that they are glued by the adhesive substance from the foot gland. This is supported by the fact that when a young ball is killed and placed in alcohol it breaks up, as if the adhesive material had been dissolved. The animals when in this ball are always nervously jerking back and forth. The ball swims freely by means of the currents from the trochal discs. If, for any reason, more of the individuals turn their trochal discs in a certain direction, the whole colony will move in that direction. That the movement of the ball is due to the summation of currents is shown by mixing some india ink with the water. It is then seen that the ball always moves in the direction from whence the stronger current comes. The ball swims with a revolving motion but is nevertheless able to move with considerable precision in a certain direction. A most important characteristic of these young balls is the fact that they react positively to light. One always finds them on the side of the dish next to the window. If they are placed in a dish in the sunlight, the balls congregate on the side towards the sun. If this region is shaded they *at once* leave it and move towards a part more strongly lighted. A considerable number of experiments showed that this reaction is very marked and constant.

The time which the animals spend in this swimming ball before settling down to form a permanent colony varies considerably, depending primarily upon the illumination. So far as I have observed, the colonies usually hatch out during the morning hours, the young ball breaking away about noon or afterwards. In no case have I seen them, under natural conditions, form a colony until evening or after sundown. As long as the dish is lighted, even by diffused light, the young rotifers continue to swim back and forth along the lighted side of the dish, striving apparently

to get as near the source of light as possible. But if the ball is placed in artificial darkness they very soon begin to form a permanent colony.

The method of forming this permanent colony is interesting. When no longer influenced by the light the ball begins to move about the dish in an apparently aimless manner. If in this wandering it chances to come against some piece of water plant or other object many of the young rotifers turn their trochal discs towards this, thus checking the progress of the ball. This reaction is brought about by purely tactile stimuli. If a clean needle is placed in front of a swimming ball, the latter will stop and the young rotifers move their trochal discs along the needle. Whether they will settle down and establish a colony seems to depend chiefly on food conditions. If the stem possesses but little débris the ball may move along it for some distance, but in most cases finally swims away. If, however, the stem has more débris attached to it the young rotifers persist in their efforts to place their trochal discs on this, probably in order to get the food particles. In this way the ball is prevented from moving away. If the ball remains in one place for a time, one or two of the rotifers will be seen dragging themselves out of the ball and moving slowly along the stem. In this manner they move a short distance and then jerk back, then start out again, each time going a little farther. Soon others come out of the ball and begin moving about, until a large number are found moving up and down the stem (Fig. 2). In moving along the plant the animals extend their bodies with the long axis parallel to that of the stem. A few of the cilia of the trochal disc and a few of those in the posterior circle appear to touch the stem and in this manner the animals "crawl" along the plant, reminding one very much of a brood of young caterpillars. But it is probable that the water currents are here as at other times the most effective agents of locomotion. In these movements there is no revolution on the long axis. In advancing along the stem the animal usually shows great hesitation in going over portions which have not been traversed by some member of the colony. In the space over which several rotifers have travelled others move with little hesitation, but on coming to new territory they move forward a

short distance and then draw back, often repeating this several times. Thus each individual pushes some distance beyond the previous limit, but at last it usually turns around and moves back towards its fellows. This like certain observations previously mentioned seems to indicate that the adhesive thread which they always secrete as they move about, furnishes a guiding stimulus for the young rotifers. Sometimes an individual leaves the stem and swims a short distance from it, always attached by the thread.

By such movements the whole ball finally breaks up and the individuals are seen moving back and forth along the stem, some-

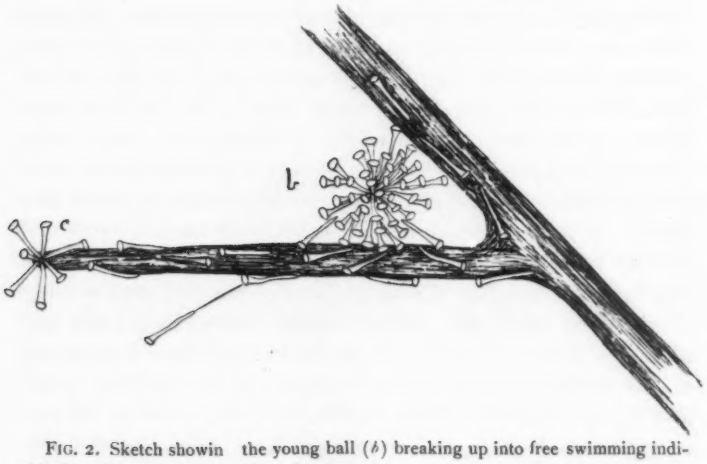

FIG. 2. Sketch showin the young ball (*b*) breaking up into free swimming individuals and the permanent colony forming at *c*.

times venturing quite a distance from the majority of the colony. But soon a few individuals settle down at some point and remain fastened by their posterior ends, with the body projecting at right angles from the stem (Fig. 2, *c*). Soon other individuals attach themselves in a similar manner with their posterior ends close to those already attached. This is the beginning of the formation of the permanent colony. In a comparatively short time all of the young animals have attached themselves in a closely aggregated group surrounding or nearly surrounding the stem. This permanent colony may be formed close to where the ball broke

up or at some distance from it. Sometimes it is formed at the end of a branch, sometimes at its base, or even on the main stalk; at other times in intermediate positions. If the swimming balls are allowed to remain in a dish containing no water plants soon after dark they begin to form a colony on the sides or bottom of the dish. Apparently when the stimulus from the light is removed the colony-forming instinct becomes dominant. When the young balls are placed in artificial darkness, colony-formation begins in from fifteen to thirty minutes and is usually completed within an hour or an hour and a half. Most of these colonies are found in well-lighted places. This doubtless arises from the fact that the swimming balls remain on the lighted side of the vessel and tend to form a permanent colony soon after this stimulus is removed. This is of advantage to the animals for they feed partly on diatoms and small protozoa which are more abundant in favorably lighted places.

Thus the young rotifers tend to remain attached in the swimming ball during their natatory life, but at the proper time they are able to sever this connection and to leave the ball one by one. Possibly the stimulus due to the contact of their posterior ends is sufficient to keep them together, until some stronger stimulus, such as food, overcomes the contact stimulus and induces them to separate. Hunger satisfied, they again respond to the stimulus of mutual contact and assemble anew, this time to form a permanent colony.

With the formation of this permanent colony changes take place both in the behavior and in the structure of the animals. Up to this time the young rotifers have behaved in a very nervous manner, constantly contracting and extending the body. But as soon as the animals settle down in the permanent colony they lose much of this irritability and remain with body and trochal disc expanded for long periods. Yet even in the adult colony one individual or another is frequently seen to suddenly fold the trochal disc and strongly contract the body, then at once begin to expand more slowly. Sometimes this contraction is evidently a reaction to some floating particle that has touched the trochal disc. More frequently there is no visible cause for the contraction. The animals in the permanent colony have another method

of reacting, not found or at least not so well marked, in the free swimming balls. When the colony is stimulated as by a slight jar to the dish or a disturbance in the water the whole colony contracts at once and if the stimulus is strong they may remain contracted for some time. This is the usual method of reacting to mechanical, chemical or electrical stimulation.

After the young animals have once formed a permanent colony they become incapable of repeating the colony formation. If some of the individuals of a permanent colony are removed from the stem they do not behave as they did previous to the formation of the colony. They swim about by means of the ciliary currents, but in an entirely aimless fashion. They neither attach themselves to the old colony nor form a separate colony. I have kept such removed individuals alive for several days but during this time they made no attempt to reform a colony.

There are likewise several structural changes which take place after the formation of the permanent colony. The animals soon show considerable increase in size, the trochal disc becomes broader and the notch on the ventral side of this makes its appearance. The small circlet of cilia at the foot of the animal disappears and the character of the cement secreted by the foot-gland changes. This cement is no longer dissolved by alcohol as it was in the young specimens. The gland is always much smaller in the adult than in the free swimming individual. But the most striking change and one which perhaps accounts for some of the changes in behavior, is the degeneration of the eye spots. As stated before, the rotifers when hatched possess two red eye spots; the reaction to light of the swimming ball is probably due to these structures. After the permanent colony has been formed for several hours one finds that the eye spots have disappeared from their previous position while two small red bodies are floating about in the body cavity. In most cases these bodies are inclosed in floating corpuscles which have considerable resemblance to leucocytes. Montgomery ('03) mentions the presence of certain non-cellular corpuscles floating in the body cavities of certain Flosculariidæ. He regards these as waste products. Since in *Megalotrocha* these bodies often enclose the degenerating eye spots and these latter are seen to

gradually waste away, it seems possible that they may function in the same manner as the leucocytes of many other animals. After the eye spots have disappeared from their original location the animals no longer react to light.

General and Comparative.

The main features in the formation of new colonies of this rotifer are then as follows: When first hatched from the eggs the young are free swimming, but do not leave the colony singly. These individuals come together into a swimming ball which reacts positively to light. Later under certain conditions this breaks up into free individuals again. These then aggregate themselves into a permanent colony in which the animals spend the remainder of their lives. In this colony formation the mucus-like secretion of the foot-gland plays an essential part.

The details of colony formation in other rotifers has apparently not been described, but it seems probable from the known facts that a similar sequence of processes occurs in some other cases. In *Lacinularia socialis* according to Huxley ('53), Hudson and Gosse ('89) and others, the process appears to resemble that in *Megalotrocha*. The young animals come together into swarms or balls and swim freely. Later permanent colonies are found on water plants. In two species of *Megalotrocha* from China, *viz.*, *M. semibullata* and *M. spinosa* described by Thorpe ('89 and '93), the adult colonies are free swimming. In these cases the eye spots are present in the adults. The new colonies swarm out of the old free swimming ones. Occasionally these balls may be found suspended from aquatic plants by mucilaginous threads. In the genus *Conochilus* we find swimming colonies of a slightly different nature. Here the free swimming balls consist of several adults with many of their young. According to Hudson and Gosse many of the newly hatched rotifers make a place for themselves in the adult ball by squeezing between the older members, while others of the new brood form new balls and swim away. There are several other more or less rare species of Melicertidæ in which free swimming or fixed colonies have been described. The free swimming adult colonies are interesting as apparently marking a step in the formation of the

fixed colonies. While the occurrence of colonies or swarms is common in the animal kingdom, I have seen no accounts of colony formation in other groups that resembles the processes seen in these rotifers.

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May 1, 1906.

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THE "ACCESSORY CHROMOSOME" IN EPEIRA.

EVIS HOWARD BERRY.

Introduction.

The problem involved in the following paper was suggested to me in August, 1905, by Professor E. B. Wilson, in view of the apparent contradiction that occurs between the insects and spiders in regard to the origin and behavior of the so-called "accessory chromosome."

In many of the Orthoptera (Acrididæ ('00), and Locustidæ ('02) McClung; Acrididæ ('00 and '02) Sutton; and Phasmidæ ('01) de Sinéty) the accessory chromosome has been found to be derived from a single, univalent spermatogonial chromosome, which splits longitudinally during the growth period, the two halves passing bodily to one pole in the first division. In the second division, however, these two halves were found to separate, being distributed equally to each of the resulting spermatids. Professor Wilson in his "Studies on Chromosomes," II. ('05), gives a general account of the accessory chromosome in the Hemiptera, which is consistent with the preceding results in the Orthoptera, though not identical. He says, p. 533: "The 'accessory' or heterotropic chromosome is certainly in most Hemiptera — and I believe will be found in all — unpaired in the spermatogonia, and its behavior is throughout that of a univalent body. . . . This chromosome divides in only one of the maturation divisions, passing undivided to one pole of the spindle in the other. The latter division is usually the second (*Pyrrochoris*, *Anasa*, *Protenor*, etc.), but in *Archimerus* and *Banasa* it is the first. In either case one half the spermatozoa receive one more chromosome than the other half."

Opposed to the foregoing conclusions are those of Montgomery ('05), obtained from a study of *Syrbula* (one of the Acrididæ), and of *Lycosa*, a spider. In these two unrelated forms he found the accessory chromosome of the growth period to be formed by the union of *two* univalent spermatogonial chromo-

somes at the time of synapsis. The mode of division of this bivalent accessory was not positively determined, but he suggested that the similarity in its formation gives some evidence that it may behave in the same way as the other chromosomes in both mitoses, dividing first reductionally and then equationally, thus being equally distributed to all of the spermatids. Nearly at the same time Wallace ('05) published her final paper on the spermatogenesis of *Agalena*, a spider, with results quite different from those of Montgomery on *Lycosa*. I will quote her brief summary, p. 182.

"1. The spermatogonia contain two accessory chromosomes and thirty-eight other chromosomes.

"2. In the primary spermatocytic division, the two accessory chromosomes pass over undivided into one of the daughter cells. The reduced number of other chromosomes is nineteen and these divide transversely.

"3. In the secondary spermatocytic division, the two accessory chromosomes again pass over undivided into one of the daughter cells. The nineteen other chromosomes divide longitudinally.

"4. Only one fourth of the spermatozoa contain the accessory chromosomes.

"5. Apparently the remaining three fourths of the spermatozoa degenerate after almost or altogether reaching maturity. In this respect they are regarded as homologous to the polar bodies thrown off by the ovum."

In view of these perplexing and contradictory accounts, I undertook the present work on *Epeira sclopetaria* to see if I could throw any light on the question. Owing to lack of material I have been able to study only one family, the Epeiridæ. The results which I have to offer on the origin and behavior of the accessory chromosome here are consistent with those of McClung, Sutton and Wilson, already mentioned, and also with those of Blackman on the myriapods ('03), but they give no explanation of the re-results of Wallace and Montgomery, which are widely different from my own. At a future time I hope to examine other families of spiders with respect to this discrepancy; certainly in none of my preparations of *Epeira* do I find any trace of degenerating spermatozoa.

I am greatly indebted to Professor Wilson for assistance in directing this work, and in the preparation of this paper. I wish also to thank Professor Calkins for his kindness in correcting the manuscript.

Terminology, Methods, Etc.

At Professor Wilson's suggestion I shall designate the "accessory chromosome" as the "odd chromosome," a name first used by Montgomery in a somewhat different sense.[1] Since there is no reason for calling it the "accessory chromosome," and since it behaves heterotropically in only one division, it seems advisable to adopt the simpler name, with the important significance that it has no mate throughout the history of the male germ cells.

Material was obtained in August and September, 1905, and also again in the early spring of 1906. The testes were dissected out rapidly in the fixing fluid, instead of the customary normal salt solution, as the process was thus rendered much easier. Strong Flemming gave the best fixation, and the finest results for general study were obtained with iron hæmatoxylin and pure saffranin, the latter giving especially beautiful and valuable results. Several different stains were tried as a differential test for a plasmosome, thionin, Auerbach and Flemming-triple. Long-extracted iron hæmatoxylin slides were studied also, but in no case could I find any trace of a plasmosome.

The figures for this paper are camera drawings made with a compensating ocular, No. 12, and a $\frac{1}{12}$ oil immersion lens. They were enlarged 2½ diameters with a drawing camera, corrected from the original, and then reduced one half in the final plates.

Spermatogonial Chromosomes.

Longitudinal sections of the testes give a complete series of stages from the resting spermatogonia around the periphery to the ripe spermatozoa in the central lumen. For this work it did not seem necessary to make a detailed study of the spermatogonia, the one important thing being to determine the number

[1] In his latest paper ('06) Montgomery recognizes the fact that there is no distinction between the "odd chromosome" and the accessory, as was pointed out by Wilson ('05). Montgomery now proposes the term "monosome" for this chromosome.

of spermatogonial chromosomes. With this in view I selected eight of the best polar metaphases of the last spermatogonial division, and drew them carefully with the camera. Seven of the

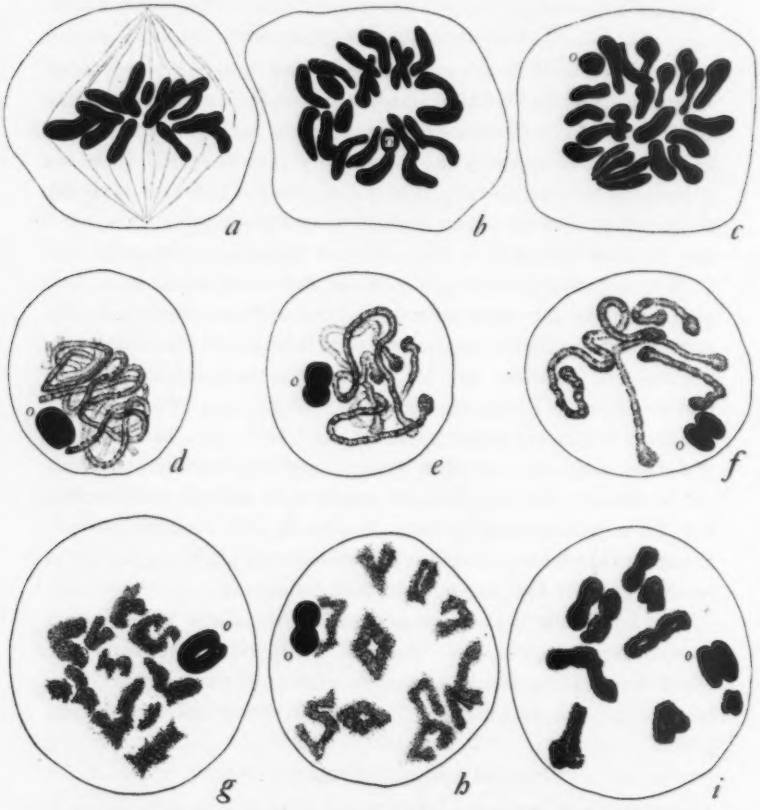

FIG. 1. *a–c*, last spermatogonial division; *a*, side-view of spindle; *b*, *c*, metaphase groups, showing twenty-three chromosomes, odd chromosome (*o*); *d–i* growth period, showing characteristic forms of the odd chromosome (*o*); *d*, contraction phase, early growth period; *e*, *f*, later stages showing the shortening and thickening of the spireme threads; *g*, *h*, late growth period, beginning of condensation; *i*, prophase of first division.

eight camera drawings gave a count of twenty-three chromosomes. In the eighth the chromosomes were so compact that I could not count definitely beyond nineteen, but from the seven

good ones it seemed conclusive that twenty-three was the correct number. Owing to the fact that the chromosomes often lie one upon the other, these counts were made with difficulty. In Fig. 1, *b* and *c*, distinct size and form differences may be readily observed. These variations are due in part to foreshortening, yet allowing for this error twenty-two of the chromosomes can be symmetrically paired off into eleven pairs, while the twenty-third is left without a mate. This is the small, round chromosome marked *O*. Since this chromosome has no mate, it can take no part in synapsis, and I infer that it is the odd chromosome, which persists as a compact, deeply-staining nucleolus throughout the growth period, Fig. 1, *d–i*.

GROWTH PERIOD.

Fig. 1, *d*, represents the contraction phase of the early synaptic period, with the ordinary chromosomes in the form of a closely massed spireme, while the odd chromosome appears as a large compact, deeply-staining nucleolus at one side of the contraction figure. Even as early as this the odd chromosome often appears double as in *f*, consisting of two closely approximated halves. This I interpret as the result of an equation-division. This double nature has been observed in other forms (*cf. Syromastes*, Gross; *Anasa*, Wilson; *Brachystola*, Sutton; Acrididæ, McClung). In the spiders Wallace and Montgomery have described the two accessory chromosomes as still separated at this period. In my own preparations I find a few cells, of the early growth-period, with two compact nucleoli, sometimes lying side by side and sometimes widely separated. At first I thought one of these might be a plasmosome, but with all of the differential stains mentioned above, both bodies took the nuclear stain equally well. These cells are of rare occurrence, and are, I think, abnormalities, resulting from this early equation-division of the odd chromosome.

In a later stage (Fig. 1, *e*) the chromosome threads loosen, and appear as longitudinally-split rods, with knobbed ends, the odd chromosome here being often bipartite in appearance. These chromosome threads shorten and thicken to form the chromosomes of the late growth period. In *g* and *h* the eleven ordinary

chromosomes have assumed the characteristic forms of rods, V's, rings, crosses, etc., preparatory to the final condensation of stage *i*.

Maturation Divisions.

In Fig. 1, *g* and *i*, the odd chromosome is seen in two of its most characteristic forms, which we encounter again and again

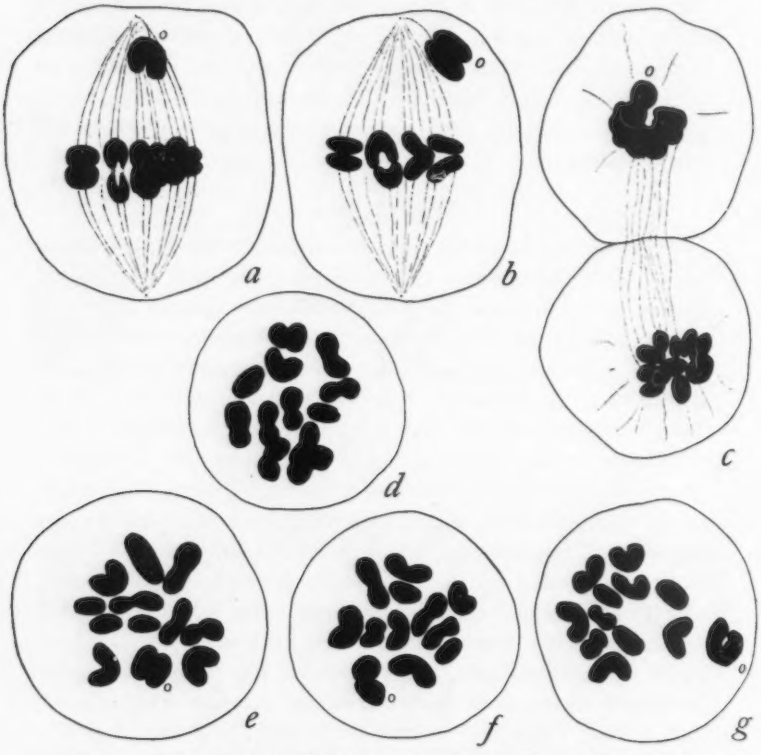

Fig. 2. First spermatocyte-division; *a*, *b*, metaphase-figures, side-view, showing odd chromosome passing undivided to one pole; *c*, telophase showing the odd chromosome present in but one of the daughter-cells; *d*, *e*, *f*, *g*, metaphase-figures, polar view, showing the odd chromosome, identified by its different level.

on the first division spindles. Fig. 2, *a* and *b*, represent such forms, attached by one spindle fiber and passing bodily to one pole in advance of the other chromosomes. These first division

figures are exceedingly numerous, the odd chromosome always being in this relation to the other chromosomes. From the similar shapes and positions of the ordinary chromosomes on the spindle, as compared with those described by other observers, I should say that the first division in *Epeira* is a reduction division and the second an equation-division, but I have not yet studied for this point and have no new evidence to offer.

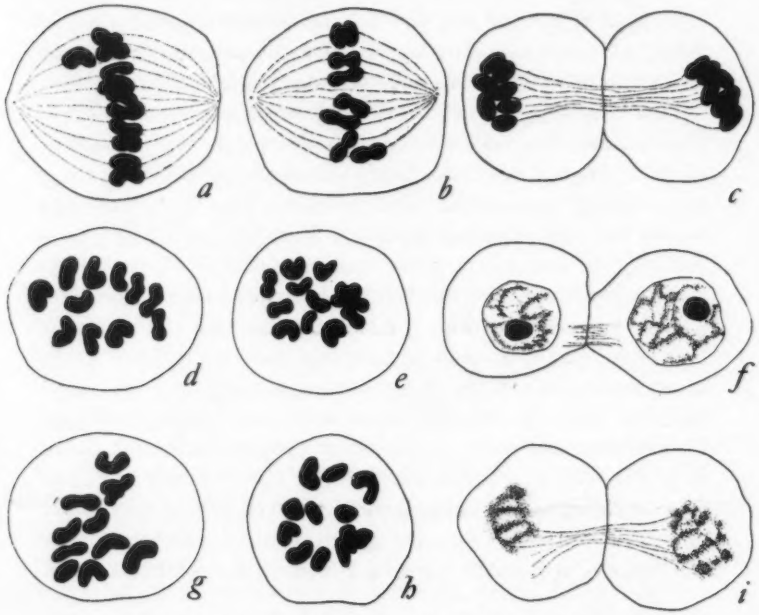

FIG. 3. Second spermatocyte-division; *a*, *b*, spindles, side-view, *c* — late anaphase; all of the chromosomes not pictured in *b* and *c*; *d*, *g*, metaphase-figures, showing eleven chromosomes; *e*, *h*, metaphase-figures, showing twelve chromosomes; *f*, *i*, telophases — *f* showing presence and equal distribution of odd chromosome, *i* showing absence of odd chromosome.

Fig. 2, *d–g*, represents first division polar metaphases showing the eleven ordinary chromosomes and the odd chromosome, *O*, which can always be recognized by its different level.

Fig. 2; *c*, represents a first division telophase, showing the odd chromosome in but one of the daughter cells (*o*, in the upper

half). In the lower cell the eleven ordinary chromosomes can be counted. Resulting from the division of each primary spermatocyte are two types of secondary spermatocytes, one containing twelve chromosomes, and one eleven. These two types divide, giving polar metaphase groups that show either twelve or eleven chromosomes (*cf.* Fig. 3, *d* and *g* with eleven, and *e* and *h* with twelve chromosomes).

In side view the second division spindles are all of exactly the same type (Fig. 3, *a* and *b*). I have studied a great many of them, and in no case do I find the odd chromosome passing undivided to one pole as Miss Wallace describes for *Agalena*.[1]

As I have suggested before, the second division is an equation-division, and the odd chromosome divides. in all probability, along its original split, thus being distributed equally to each of the resulting spermatids. The telophase, Fig. 3, *f*, represents two of the early spermatids derived from the preceding type of division, the odd chromosome still recognizable by its definite shape and deep-staining reaction. From the division of the other type of spermatocyte Fig. 3, *i* (without the odd chromosome), are derived two spermatids without this dark nucleolus. There are, therefore, two types of spermatids existing in equal numbers, one half with the odd chromosome, and one half without. To establish this point, I counted in forty-four different fields, all of the early spermatids with the odd chromosome, and all of those without. This count gave a total of 287 of the former and 286 of the latter, thus giving very positive evidence that the two types of spermatids exist in the proportion of half to half.

Summary.

1. The spermatogonia contain twenty-three chromosomes, twenty-two ordinary ones and the odd chromosome.

2. The odd chromosome is the chromosome-nucleolus of the growth period.

3. In the first maturation-division the odd-chromosome passes

[1] It seems possible that Miss Wallace may have mistaken a first division spindle for a second-division. Her two types of second-division spindles, Figs. 27 and 28, Plate IX., vary considerably in size, and by comparison of the larger, Fig. 28 (the one with the accessory chromosomes), with Fig. 18, it could, I think, easily represent a first division.

undivided to one pole, the eleven ordinary chromosomes dividing by a reduction-division.

4. The second maturation division is an equation division, in which the old chromosome participates.

5. Resulting from the first division are two types of secondary spermatocytes (1) containing eleven chromosomes, plus the odd chromosome, and (2) containing only eleven chromosomes.

Resulting from the second division are two types of spermatozoa (1) containing eleven chromosomes, plus the odd chromosome, and (2) containing only eleven chromosomes. These two types of spermatozoa exist in equal numbers.

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May 16, 1906.

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THE FORMATION OF PROGLOTTIDS IN CROSSOBOTHRIUM LACINIATUM (LINTON).

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I. Introduction and Summary.

The statement that all cestodes form their proglottids by the insertion of each new one between the scolex and the one last formed, has been current in our text-books for years and so far as I know there are no statements to indicate that any other mode of origin ever occurs. Upon turning to the original literature, one finds that the above statement rests upon the descriptions given about 1850 by Leuckart for the genus *Tænia* and closely related forms. This account was confirmed and supplemented by other authors about the same time, but in recent years we only find the process referred to as something established beyond all question as the universal method by which proglottids originate. No recent work so far as I am aware attempts to call this dogma in question, nor do writers longer think it worth while to record the fact that they have confirmed so well established a phenomenon.

Despite this universal acceptance our current description appears to be based upon published accounts which deal with a limited number of forms, though the fact that these accounts have not been contradicted leads one to believe that they describe the process correctly in those species which have been most frequently the subject of investigation.

The cestode, *Crossobothrium laciniatum*, a species occurring as an adult in the "sand shark," *Carcharias littoralis*, of our North Atlantic coast, does not conform to the current account in its method of forming proglottids. In this cestode there first appear from behind forward a considerable number of proglottids (Figs. 1, 2 and 10 of Pl. IV.). These "posterior" segments, as I shall call them, continue to form after the accepted fashion until about 35 of them have been produced. They are marked off by straight transverse lines and not until a considerably later period

do even the oldest of them begin to show the sculpturing of the posterior margin which is so characteristic for the adult.

When these posterior proglottids are about 35 in number, there begin to appear, in the unsegmented region just back of the scolex, others which may be termed the "anterior proglottids." These are produced in the reverse order, *i. e.*, the youngest is the most posterior one.

From such a condition as this (Fig. 10, Pl. IV.) the formation of proglottids continues so that from this time on we may say that the body is segmenting from both ends toward the middle. This process gradually obliterates the unsegmented zone (Fig. 12, Pl. V.) but specimens several centimeters in length may still show such a "zone" at a point near the line dividing the anterior and middle thirds of the body.

When this zone finally becomes obliterated there have been formed in the neighborhood of 50 "anterior" proglottids and upwards of 400 "posterior" ones. With this obliteration of the zone we reach what may be termed the fully formed adult condition as is shown by Fig. 14, *a–n*. *After such a stage is reached no more proglottids are formed.* and the specimen remains unchanged save for the continual maturing and separating off from the posterior end of the motile proglottids. This latter process finally reduces the total number of the segments to such an extent that the condition is perceptible at first glance. Such a specimen which had only 96 proglottids is shown in Fig. 16, *a* and *b*.

When the number of units in the strobilla has been reduced somewhat further, the region between the scolex and the first proglottid begins an active growth and elongates into a neck region as is shown by Figs. 21 and 15. After further elongation and the continual separation of the terminal proglottids, this neck region begins to form segments while there are still some of the most anterior segments of the old chain remaining (Figs. 17 and 24). This new segmentation begins as does that previously described by the appearance of posterior segments and later of anterior ones, and although I have no stages beyond the point shown in Fig. 24, I think one is justified in the opinion that this segmentation is the beginning of a new chain and that soon a new strobilla will be formed which would be with difficulty, if at

all, distinguishable from the one proceeding from the first segmentation. The specimen represented by Fig. 19, Pl. VI., is an exception to the above course of development, in that it shows all the characteristics of a specimen having the non-segmented zone of the early proglottid formation and at the same time an elongated neck. A possible explanation of this as a specimen undergoing regeneration after mutilation is given on p. 215 of this paper.

A discussion of the bearing these facts have upon our views regarding the nature of the cestode body in given on p. 217.

II. Discussion of the Literature.

In my examination of the literature I have relied upon Braun's monograph (Bronn's Thierreich) as a trustworthy summary of the facts established regarding the Cestoda down to about 1896, when the parts of this volume referring to the points in question appeared. Upon the particular subject of the method of proglottid formation the statements of this author are explicit in several instances and agree with the account which has long been current in our text-books. For example, in the introductory section on Cestoda (p. 1167), he says: "besitzen dem nach die Cestoden Zweierlei Theile: Scolex and Proglottiden: letzterer sind in einer Reihe dem Alter nacht geordnet, so das die jüngsten dem scolex am nächsten, die ältesten von diesem am weitesten entfernt sind." On p. 1224, under the paragraph entitled "Intercalation und Verwachsung von Proglottiden," Braun says: "wir haben allen Grund zu der Annahme dass die Proglottiden die ein Bandwurm besitzt oder jemals besessen hat, alle nach einander und zwar in der durch die Stellung gegebenen Reichenfolge am Scolex sich gibildet haben, dass also niemals durch einschalten neuer Proglottiden die Zahl der Glieder an einem Bandwurme vermehrt wird." Again, under the section of the Embryology entitled "Die Uebertragung der Finnen in den Endwirth," etc. (p. 1592 on), he refers in a number of places to the appearance of the segments, but makes only indirect mention of the order in which they appear, the evident reason for this being that there is only one method of proglottid formation known.

On page 1597, Kuchenmeister, von Siebold, Lewald, Haubner and Leuckart are quoted as having studied this stage of the development in a number of forms about 1850, since which very little attention has been paid to it. Leuckart's first work upon this point appeared in 1856 and was subsequently elaborated in the editions of his text-book.

On p. 1600, Braun says: "Die näheren Vorgänge bei der Bildung der Proglottiden sind uns bisher noch ganz unbekannt, nur die Anlage und Entwickelung der Genitalien ist bei manchen Arten erforscht."

From indirect references at other places in the volume it is clear that the accounts current in our text-books are justified by the absence of any published description contradicting the accepted method of proglottid formation.

The amount of literature published on Cestodes up to 1896 is enormous, but the manner of proglottid formation was established and had been explicitly described for so long a time before this date that one can hardly believe any marked exception to the universal method could have escaped the notice and comment of so thorough a reviewer as the author of this monograph has shown himself to be. Upon such a point as this I feel that Braun may be taken as reliable and that I am justified in the conclusion that no such process of proglottid formation was described previous to 1896.

I have consulted a considerable amount of the literature on Cestoda appearing since that date, at first hand, and have cov ered the abstracts and titles of cestode papers as they appear in the *Zoölogische Jahresbericht*, without finding anything to indicate that accounts have been published of a method of proglottid formation other than the one commonly accepted as the universal.

Papers upon other phases of the proglottid question have appeared, as for example one by Lühe, '98, who, in his examination of the segmentation of *Ligula*, found that the segments which occur at the anterior end do not correspond to the arrangement of the genitalia where these occur toward the posterior end of the segmented area and hence, that this case is not one of true proglottid formation but rather to be considered as a differentiation of another sort. While there may be some homology

between these pseudo-proglottids of *Ligula* and the "anterior" proglottids of *C. laciniatum*, I have made it clear that the greater part, if not all, of these structures appearing at the anterior end become true proglottids in the form I studied.

Instances of irregularities like the occurrence of half proglottids are noted by Braun, p. 1225, and dismissed as abnormalities. Such a condition has recently been discussed by Child ('02) for *Moniezia*, but this again has nothing to do with the points I have brought out for *C. laciniatum*. The fact that no exception to the typical method seems to have been recorded is to my mind justification for an extensive account of the mode of proglottid formation which I have observed in *C. laciniatum* and such an account is therefore given in the section which follows.

With the exception of the work by Linton, who described the species, I know of nothing which has been published upon *C. laciniatum*. This author in his original description (Linton, '86, p. 474, Pl. III.) speaks of small specimens and of the segments occurring at either end in somewhat larger ones. The posterior segments are, he says, "totally unlike those of the adult" and "are evidently evanescent." It is, therefore, likely that Linton had among his specimens the various stages which I have described, but he paid little attention to them and hence his brief paragraph does not bring out their significance.

The "long-necked" specimens were also observed by Linton and are referred to on several occasions. In his "Report of Parasites Collected in 1898" (Linton, '99) he records several instances, and in an earlier paper ('89, on pp. 800–801) and Pl. VII., Fig. 4, he describes the "long-necked" forms and others with a reduced number of segments in such a way as to make it clear that he has seen all the stages up to the point where the segmentation of the neck region begins. While not thinking that they justify the erection of a new species he speaks of them as a variety (longicolle) of *C. laciniatum* and suggests that they may represent the transition to another species. It is thus evident that Linton being interested primarily in the systematic work, did not examine this point sufficiently to ascertain the real significance of such stages. The importance of his observations for my work is that they confirm my statement that a certain pro-

portion of long-necked specimens are always found when any large number of the individuals of this species is examined. This is borne out by the observations of some of my colleagues on the staff of instruction at the Marine Biological Laboratory, who have frequently observed such individuals among the specimens used by the students. In this connection it may be said that all the specimens which have been examined by these men, by Linton and by myself have been taken between June 15 and September 15. Since the sand sharks are not present about Woods Hole from November to June (Smith, '97, p. 89) one could not obtain data throughout the year. It would, however, be desirable to examine the worms from sharks taken as late and as early as possible, for there may be seasonal relations such as obtain in the period of maturity in other forms,[1] and one might find at another period a relatively larger number of "long-necked" forms.

III. Detailed Account of the Proglottid Formation.

(*a*) *Material and Technique.* — The *C. laciniatum* from which the data here presented have been obtained were collected at Woods Hole, Massachusetts, during several summers (1902–'04). I have examined a large number of specimens which have accumulated since I began work upon this form and by selecting out the different stages have been able to demonstrate every step in the history of the strobilla with an abundance of material. In some cases, the specimens were killed under the pressure of a cover slip, but for the most part they were taken from lots which had been killed by placing for a moment in fresh water and immediately transferring to the killing fluid. All the material used in this study was fixed in saturated sublimate with 5 per cent. acetic. Almost any good stain will bring out the division lines between proglottids, but for determining the location of the reproductive organs in whole mounts Partsch's alum cochineal gave most excellent results.

(*b*) *Proglottid Formation in the Larval Worms.* — Out of an abundance of material, I have been able to demonstrate again and again that, in the case of young worms which have presum-

[1] See the remarks of Braun, p. 1461, upon the "time of reproduction," etc.

ably just entered the shark, the proglottids originate as follows: The young worm which is recognizable by the small size and the proportions of its scolex region (Fig. 1, Pl. IV.) at first forms segments from behind forward in the typical cestode fashion (Fig. 2, Pl. IV.). The youngest specimen I have found (Fig. 1, Pl. IV.) appears to have something missing from the posterior end, but a comparison with Fig. 2 which is a little older would indicate that not more than one proglottid has been lost. The progress of this early segmentation, the increase in size and the changes in the proportions of the scolex parts are seen by comparing Figs. 1, 2 and 5, which are all drawn on the same scale. The terminal proglottid in such stages is invariably long as in Figs. 2 and 4, but in older specimens (Fig. 7) which show no signs of having lost any segments at the posterior end one is very likely to find it much shorter. In Fig. 3, for example, there is a very long terminal proglottid which is bent twice in places which are clearly not lines of division between segments. Figs. 4 and 6 represent specimens in which the most posterior line of division was much fainter than the lines anterior to it. This suggested the possibility that the last two proglottids in such cases had originated by the division of an earlier single terminal proglottid. I have often found specimens which suggested very strongly that such a division of the earlier proglottids had taken place, but since it is well-nigh impossible to make certain of such a process when specimens can only be taken and arranged in series after being studied, I do not wish to say more regarding this than that such cases as shown above have frequently come to my attention and suggested the possibility of the subsequent division of the elongated segments found at the posterior end in very young worms. The formation of simple rectangular segments from behind forward progresses steadily (Figs. 6 and 7) until from 40 to 60 of these "posterior" proglottids have been developed. There then begin to appear in the region just behind the scolex other proglottids. These "anterior" proglottids are from the first different in shape from the ones at the posterior region and this difference is, as will be shown, continued into the adult. The most important fact about these "anterior" proglottids is that they are differentiated from in front backward. Fig. 10 shows a specimen in which a number

of these "anterior" proglottids have appeared, Fig. 7 one in which there are a few more and the differences between the proglottid outlines is more apparent. These two figures moreover illustrate the absence of a very definite relation between the number of posterior segments formed and the time at which the anterior ones begin to appear, for in Fig. 7, there are 56 posterior and 10 anterior as against 66 posterior and in 6 anterior in Fig. 10. The marked differences in contour are better seen a little later, when there are more anterior segments present. Fig. 8 illustrates this and shows that the oldest ones have already assumed the shape so very characteristic for the adult proglottids of this region, and even in those which are just appearing (Fig. 8) the suggestion of the four lappets which occur on the posterior margin can already be seen. The contour of segments from this region is seen again in the figures of Pl. VI. and a stage which is practically the adult is shown in Fig. 25, *a*, Pl. VII. The four lappets are very mobile in the living specimen and their appearance differs considerably according to their state of contraction and the angle from which the strobilla is viewed as will be apparent from a glance at Figs. 14, *a*, Pl. V., 16, *a*, Pl. VI. and 25, *a*, Pl. VII.

The "posterior" segments have in their final condition four flattened lappets (Figs. 14, *n*, Pl. V., and 25, *c*, Pl. VII.). This sculpturing is distinctly different from that of the "anterior" segments until the latter come to occupy the terminal position (Fig. 15, Pl. VI.), when they assume the contours of typical posterior proglottids.

The formation of the posterior segments continues rapidly — (Figs. 10, Pl. IV., and 12, Pl. V.) and this region presents an appearance identical with that ordinarily met with in the cestode strobilla. The anterior proglottids develop more slowly and their total number is always much less than the posterior ones, hence, the region where the two meet and where the non-segmented zone is finally obliterated is well towards the anterior end.

The total number of posterior segments can be determined only approximately because there is always the chance that some have been lost either under natural conditions or during the handling incident to collection and preservation. Taking the maximum

number in the longest specimens it appears that there may be formed upwards of 400.

In the case of the anterior proglottids the total number formed can be ascertained with more certainty for it is only necessary to count them in specimens where the zone is just being obliterated. Such counts show that there may be as many as 604 proglottids formed in the anterior region. I have found a few cases in which the zone seemed about to be obliterated when there were only 30–35 anterior segments, but in the great majority of such cases their number runs well up toward 60 and I think 50 + would be a fair statement of the average number.

The transition of the posterior segments into the unsegmented zone is always gradual, as will be seen in Figs. 13, Pl. V., 23 and 25, *b*, Pl. VII. On the other hand the anterior segments may show a gradual transition into the zone (Fig. 13, Pl. V.) or the transition may be more or less abrupt (Figs. 23 and 25, *b*, Pl. VII.) The condition shown by Fig. 25, *b*, is the more common one for the anterior proglottids while that shown in Fig. 13, Pl. V., is typical for the transition of the posterior ones into the zone.

When the zone is finally obliterated it is no longer possible to tell where it was located for the proglottids of the anterior end, with their four-pointed lappets, gradually change into the straight transverse lines which separate the many immature proglottids in the middle region of the chain. The adult condition thus obtained is represented by Figs. 14, *a–n*, in which typical regions of the body have been drawn and the length of the chain between such regions shown by straight lines. The peculiar anterior proglottids (Fig. 14, *a*) still show their characteristic outlines as far back as the 110th proglottid from the scolex (Fig. 14, *c*) from which we may conclude either that the specimen figured had formed a greater number of anterior proglottids than I estimated as the average, or that some of the proglottids formed behind the non-segmented zone have assumed the character of immature anterior proglottids. From the condition shown at the 110th segment of Fig. 14, *c*, we reach after a distance represented by the line *d* a region of very close-set immature proglottids which are separated by straight transverse lines without any sculpturing

of the margins. This condition is continued for a distance shown by the line *f*, before the rudiments of the reproductive organs are clearly discernible. When a place is reached where these can be definitely made out (Fig. 14, *g*) it is seen that the proglottids are beginning to assume the outlines characteristic for the posterior members of the chain. In *g* of Fig. 14, we find the reproductive organs well marked out at the region just in front of the 386th proglottid. They are first apparent in the whole mount at about the region of the 325th proglottid. From the region shown by *g* of Fig. 14 to the posterior end the transition along *h* and *i* to the 471st at *j* is gradual and from this latter point through *k*, *l* and *m*, to the terminal region, shown by *n* of Fig. 14, the change consists more in the elongation of the proglottid than in its growth in bulk. The 506th proglottid which terminates the chain shows fully developed reproductive organs and some eggs accumulated in the uterus and is farther advanced than many of the free motile proglottids one finds in the intestine.

(*c*) *The Reduction of the Primary Strobilla and the Formation of the "Long-necked" Stage.* — Such a specimen as the one just described may be termed *a young adult*, for it still has about the number of proglottids which we have reason to believe is not far below the maximum and it is already liberating ripe segments from the posterior end. The following points are of importance in this connection: First, the reproductive organs do not begin until well back of the 300th proglottid. Though sections might show them being laid down in front of this region, there is in any case a long region extending beyond the anterior half of the worm in which they have not yet appeared. Second, the non-segmented zone has been obliterated and the place where it disappeared can no longer be recognized. Third, there is no point in the chain where there is anything to suggest the interpolation of new segments. I have examined a large number of specimens in this and in the stages next succeeding without finding the slightest indication that new proglottids are added in the region back of the solex or elsewhere.

The only change which takes place in such a specimen is the continual dropping off of ripe proglottids from the posterior end and the steady advance toward the scolex of the region of ap-

pearing reproductive organs. Specimens are found in all stages intermediate between Fig. 14 and those which like Fig. 16 have a much smaller number of segments. It thus seems clear that such a specimen as is shown by Fig. 16, *a*, *b*, is to be regarded as derived from the "young adult condition" of Fig. 14 by the progressive loss of ripe proglottids and the advance anteriorly of the differentiating reproductive organs. In the specimen just cited (Fig. 16, *a*, *b*) there is a total of only 96 proglottids in the chain and the terminal ones are ready to be set free. The reproductive organs which in the whole mount are recognizable in the 26th proglottid have advanced beyond the point (30th) which we have fixed as the very minimum for the number of "anterior proglottids." There is as yet no sign of the appearance of new proglottids to make good those which have been shed. We might refer to this as an adult which is approaching the old age period of the strobilla in contrast to such a "young adult" as Fig. 14 shows.

Specimens with a smaller number of proglottids and showing the posterior ones well matured have been frequently observed, but when the number has been reduced much beyond this point (Fig. 16) one finds an important change occurring in the region between the first of the old "anterior proglottids" and the scolex. This change consists in the growth of this region into what I shall call a "neck" which soon separates the scolex and the first proglottid by a very appreciable distance (Fig. 21, Pl. VII.). In this figure the reproductive organs are recognizable in the 17th proglottid and probably occurred further forward but the specimen from which this was drawn was poorly stained for this point. Such a specimen as Fig. 15, Pl. VI., shows the reproductive organs well established in the 5th segment while in the 10th and last one they are fully matured. Such a case as Fig. 18, Pl. VI., has clearly come from an old chain which has formed a "neck" to which two of the old anterior proglottids are attached. It is very possible, however, that this specimen which I found among many other preserved specimens has had some segments detached in the collection or subsequent handling.

Specimens in which the neck has become well established next show a segmentation at the posterior end of the neck region (Fig.

17, VI., and 22, VII.), and this segmentation progresses from behind forward as did the formation of "posterior segments" previously described. When from six to twelve such segments have been established there begins a segmentation of the front end of the neck. This proceeds from in front backwards as did the formation of "anterior" proglottids at an earlier stage. Specimens showing the above changes are shown in Figs. 17, Pl. VI., 22, Pl. VII., 20, Pl. VI., and 24, Pl. VII. The number of proglottids of the old chain which are present when the neck begins to appear and later the number present when the segmentation of the neck begins, appears subject to a considerable variation. For example, Fig. 15, Pl. VI., and Fig. 21, Pl. VII., are specimens which when the state of contraction in each case is considered appear to have the neck developed to about the same extent, but the number of remaining proglottids is ten in one case and thirty-four in the other. Nothing can be determined on this point from Figs. 17 and 18, Pl. VI., or 22 and 24 of Plate VII., for although Figs. 18, 22 and 24 had the number of proglottids indicated when they were taken from the shark they may have been mutilated when the valve was cut open, while in the case of Fig. 17 I have no record of the condition when collected and hence suspect the possibility that some segments may have been detached by accident. Again, in Fig. 20, Pl. VI., is shown a specimen in which the neck has both anterior and posterior segments though forty-nine of the old proglottids remain. This is the maximum number of the old segments of which I have ever found upon a necked specimen. It would seem, therefore, that the neck region appears when the supply of proglottids comprising the earlier chain has become so much reduced that the developing reproductive organs begin to appear well into the region occupied by proglottids which must have had an "anterior" origin in the embryo, but the number of primary proglottids remaining when the neck appears is quite inconstant.

In the early part of my work, when I had not obtained so complete a record of the conditions in the "necked" specimens, I was inclined to question whether the segments formed at the anterior end in the larval stage (Fig. 8, Pl. IV.) were to be regarded as proglottids in the same sense as those developed posteriorly,

i. e., whether we had not to do with what one might term pseudo-proglottids as distinguished from the true or posterior proglottids. This led me to consider the criterion by which one would determine whether a region of the body was a proglottid or not. My view is that we are justified in applying the name proglottid to any region of the body which is set off from the neighboring parts by constrictions and which contains a complete set of reproductive organs. From many specimens which were examined when the non-segmented zone was almost obliterated, I found as previously stated that no specimens observed showed less than 30 + segments of "anterior" origin and that the number produced in this region was 50 + in the majority of cases. If this is so, specimens like Figs. 15, Pl. VI., and 21, Pl. VII., show us that such "anterior" proglottids may eventually become sexually mature and be shed off the same as any other in the chain. Moreover, as will be seen in the two figures just cited, anterior proglottids thus becoming ripe change their shape by the flattening of the four-pointed lappets of the earlier stage and assume exactly the shape characteristic for a ripe proglottid of posterior origin. I think, therefore, that we may dispose of any question as to whether the segments originating from in front backwards are to be considered as true proglottids.

The new segmentation appearing at either end of the neck region is identical with that which occurs in the young worm save that the "anterior" proglottids begin to appear before so many of the posterior ones have been developed. Were it not for the single terminal segment and for the slightly greater size of the bothria on the scolex one would certainly class specimens like Figs. 22 and 24, Pl. VII., as young worms similar to those shown in Figs. 7 and 10, Pl. IV., and 11, of Pl. V. The size of the bothria would not be very reliable in preserved specimens, but the single terminal proglottid with its four-pointed lappets would seem unquestionably to be the first anterior proglottid of a preceding strobilla.

I have not been able to secure stages showing that the segmentation of the neck region finally results in a new strobilla. The difficulty in distinguishing specimens, which have passed farther along the way to this condition than Fig. 24, from stages in

the primary strobilla would be considerable unless one of the old anterior proglottids still remained and I have not felt that the prospect of finding such stages justified my delaying the completion of this paper until I had another opportunity to examine a large amount of fresh material in search of them. This segmentation of the neck region is either the beginning of a new strobilla which to distinguish from the earlier we might call the secondary strobilla, or it is simply an abortive attempt in the old age of the individual and comes to naught. From what we know of the longevity and continued production of proglottids in a few other cestodes, Braun, p. 1604, I think the presumption is distinctly in favor of the former view, *viz.*, that the segmentation which I have observed beginning in the neck region is the first step in the formation of a new strobilla. Though it would be almost impossible to demonstrate the formation of other strobillæ from new neck regions after the secondary one has in turn become exhausted, we may, I think, consider such a process as not at all unlikely. Hence, we have the suggestion that what may be called primary, secondary and tertiary, etc., strobillæ are successfully headed off by a single individual scolex.

(*d*) *An Exceptional Condition, Perhaps to be Explained as Regeneration after Chance Mutilation.* — Two specimens have come under my observation which apparently do not find any place in the above scheme of development. One of these is shown in Fig. 19 of Pl. VI. In this we have a body with a definite zone region and the anterior and posterior segments which characterize a worm developing the primary strobilla. In contradiction to these features, we find a well-developed neck region. The size of the bothria gives no trustworthy evidence of the age in these specimens. I think these two exceptions may be explained as cases of regeneration after a chance mutilation occurring under natural conditions.

It will be noted if Fig. 19, Pl. VI., is compared with Fig. 12 of Pl. V., that the number and development of the anterior proglottids is relatively much greater in Fig. 19 than we find in the normal development of the primary strobilla. If such a specimen as Fig. 11 or Fig. 12, Pl. V., had been cut in two somewhere near the forward region of the posterior proglottids and

by this loss of a considerable portion of the young strobilla, the part having the scolex had been stimulated to form a neck for the production of more segments, we should have such a condition as Fig. 19 shows. It is commonly assumed in the theories regarding the nature of the cestode body that the Cestoda have great powers of regeneration. For example, Lang ('88) used this supposed capacity for regeneration as the starting point in his explanation of how the strobillization was first introduced into cestode phylogeny. We have, however, little in the way of direct evidence either from observation of specimens regenerating under natural conditions or from accounts of their regeneration under experimental control. The difficulties of technique have so far as I know prevented any experimental confirmation of this supposed power of regeneration. Still the assumption seems reasonable and the difficulties could doubtless be overcome if it seemed worth while to determine what regenerative powers the Cestoda do actually possess. In the case of *C. laciniatum* one should be able to test the explanation I have just given for these exceptional cases by the mutilation of young specimens in the way indicated. If one could overcome the difficulties and keep the worms alive by introducing them into another shark or otherwise, I think some very interesting data might be obtained by the mutilation of strobillæ in different stages and a study of the regeneration if such a process occurs.

(*e*) *Histology of the Non-Segmented Zone in Young Worms and of the Neck Region in Older Specimens.* — I have examined sections of this region which is the place where differentiation appears to be most active hoping to find some characteristic features by which its histology might be distinguished from that of the ordinary proglottid, but with the material available have not been able to recognize any such distinguishing features. If nuclear division is in progress it must be amitotic such as Child ('04) has recently described for *Moniezia*, for the material I have would show clearly the existence of mitotic figures. I have endeavored by staining after the method followed by Child to determine the presence or absence of such division but the fixation is not quite good enough for this point. This histology seems to be much the same as that of the formed proglottid save that

the muscle fibers are less developed. In the young worm the zone has a circular outline in section while in the neck region it is somewhat flattened. Fig. 26 which represents the structures found in this zone of a young worm shows that there occur only the structures which are regarded as typical for the adult cestode (Schneider, '02, p. 311, Fig. 324, and Braun, Pl. XLVII.). There are circular and longitudinal muscle fibers between the cuticle and the palisade of subcuticular cells, the latter being grouped so that they form lines between the outer portions of these cells. The parenchyma contains scattered nuclei, some with cytoplasm aggregated about them as in a definite cell body. One member of each pair of water tubes appears in the figure. The results of this examination are disappointing, for unless there is an active amitotic division, which I fail to make out with this material, there is nothing which would suggest the active differentiation which is taking place. However, I do not offer this as in any way an adequate discussion of the histology of this region. Such an account I hope to give at some future time when I have proper material for a thorough examination. This is merely a statement of what I have been able to accomplish along this line thus far.

IV. General Considerations.

If my interpretation of the stages found is correct, we have in *Crossobothrium laciniatum* a method of proglottid formation which is radically different from the one now accepted as universal. Also, there seems to be more than one strobilla formed so that we may speak of primary and secondary strobillæ and perhaps there are even more. This is in marked contrast with such forms as the tænias which present a continuous growth and differentiation in the neck region to make good a continuous shedding of ripe proglottids. If we try to analyze the proglottid formation here described and to compare it with the process which occurs in other cestodes, we may do so in the following way. The appearance of the first proglottids at the posterior end is homologous to the method now accepted as the universal one. Such a stage as is shown by Fig. 5, Pl. IV., and from this on to the time when the "anterior" proglottids begin to appear, may

be regarded as a cestode with an unusually long neck region. The essential point of difference comes when proglottids begin to differentiate at the anterior end of this neck *in the reverse direction.* It must however be noted that subsequently the front part of this neck region becomes the neck of the next strobilla which at its posterior end differentiates from behind forward. It is thus possible to regard the segment formation of *C. laciniatum* as derived from the ordinary type by the acquisition of a long neck and subsequently its differentiation in the reverse order at the front end.

While it is clear that the proglottids of the adult are serially homologous throughout the worm, we may ask ourselves whether there can be found any good reason for the modification of structure which is so noticeable in those of the anterior region. If we consider the case of such a "young adult" specimen as that shown by Fig. 14, *a–n*, Pl. V., it is clear that this modification of the four posterior corners into sharp projections is not a characteristic belonging exclusively to the proglottids which had an anterior origin. In this figure if we examine *a*, *b* and *c* we find that the area occupied by proglottids having the shape in question extends considerably beyond the region occupied by the proglottids which probably had an anterior origin. In such a specimen we can no longer determine where the non-segmented zone finally disappeared, but as has been pointed out there are never many more than 60 anterior ones and hence the zone must have been not far from the 60th segment. The proglottids having the shape in question are found extending even back of the 110th as shown in *c* of Fig. 14. This means when applied to such a figure as 13 of Pl. V., or 23 of Pl. VII., that some of the forward members among the simple proglottids behind the "zone" are later made over into the anterior type, and in Fig. 25, *b*, Pl. VII., we see that this may begin even before the zone is obliterated. It is thus clear that the fact of a proglottid having an "anterior" origin is not the factor which has determined the peculiar shape of the segments at the front end of an adult *C. laciniatum*, but that the antero-posterior differentiation which exists extends beyond the region of the obliterated "zone" and into that of the posterior segments. The cause would then seem

to be something which affected the anterior end of the strobilla independently of the origin of the proglottids involved.

It has occurred to me in collecting the worms from the spiral valve that perhaps the four projections which characterized these forward segments of the adult strobilla, may be regarded as an adaptation by which the worm is better able to retain its hold with the scolex. The passage along between the folds of the semi-solid feces which are often found in the valve, must often draw out the bodies of these cestodes and tax the hold of the scolex to a corresponding degree. The projections in question would be very effective in preventing the front part of the worm from slipping backward and would thus relieve the strain on the scolex. I believe that we must look to something in the conditions which act upon the anterior region of the strobilla and not to the anterior or posterior origin of any given proglottid, to account for this feature of *C. laciniatum* and the above suggestion is given as a possible explanation.

Since the theories regarding the nature of the cestode body have taken into consideration the supposed universal method of proglottid formation we may consider what bearing the facts here established have upon such theories. At the outset I will say that I do not think this single case of a small number of true proglottids developed anteriorly and in the reverse direction will justify any sweeping modification of existing views, for it is only fair to suppose that the posterior proglottids of *C. laciniatum* are strictly homologous to the entire strobilla in other cestodes and we may regard *C. laciniatum* as a species in which another method of proglottid formation has been superposed on an older and more universal one. The history of our theories regarding the nature of the cestode body is extensively summarized by Braun in the Cestode volume of Bronn's Thierreich. Beginning with the earlier conception of the chain as composed of proglottids which had become united, he traces the history of the theory developed by J. P. van Beneden, Siebold, Leuckart and others, which viewed the Cestode at a "polyzoötic" organism, *i. e.*, that despite the physiological unity of the chain of proglottids we are dealing, from the morphological standpoint, not with a unity but with an animal stock. This view has been main-

tained by some to the present day, having been defended more recently by J. v. Kennel in his "Lehrbuch der Zoölogie." On the other hand it has been subject to considerable attack which is summarized in the theory accepted by Lang and Korschelt and Heider. The latter authors hold that the scolex and a single proglottid represent the individual and are equal to the body of a trematode and that the present organization of the cestode body has been attained by the reduplication of that region which contains the reproductive organs. In a way this opinion is not so different from the other view since we may think of this reduplication as a budding process in which only the posterior half of the individual is formed.

Broadly speaking, the discussion reduces itself to the question whether we shall consider the segmented condition of the cestode as having arisen by the loss of individuality and the adherence together of originally complete individuals which were formed by budding, or as arisen by the elongation and reduplication of certain parts in a single individual. It is like the question of metamerism which though most commonly explained in accordance with the latter (Lang, '82, and others) has nevertheless been accounted for by some (Whitman, '99) along the line of the former view. Personally, I agree entirely with Braun in the concluding paragraph of the discussion above cited, that the theory one will incline to is largely a question of temperament since the phenomena can be interpreted in either way. The facts brought out for *C. laciniatum*, while they show a new method of proglottid formation do not turn the scale in favor of either interpretation. If such a condition as is here described could be shown to be more widespread in the cestoda and thus the one we now regard as primitive should lose some of its importance we might then believe that a segmentation proceeding from both ends toward the middle is better explained upon the hypothesis that reduplication of parts has occurred in a single individual than upon the theory that it has arisen through a process of strobillization identical with that by which the ephyræ of a scyphozoan are formed. But the facts regarding *C. laciniatum* stand alone and we can at present claim no more than that the formation of proglottids in the cestoda deserves renewed investi-

gation particularly in any forms which suggest a departure from the type of development hitherto supposed to be without exception.

ZOÖLOGICAL LABORATORY, UNIVERSITY OF MISSOURI,
COLUMBIA, MO., June 15, 1906.

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PLATE IV.

Figures showing stages in the proglottid formation of *C. laciniatum* as found in the spiral valve of the shark, *Carcharias littoralis*. All from camera lucida outlines. The magnification is given for the figures as reproduced.

FIG. 1. The youngest specimen obtained. The outline at the posterior end indicates that at least *one* proglottid has been lost, but the small size of the scolex shows that this one is slightly younger than the complete specimen shown in Fig. 2 and hence it is unlikely that more than one proglottid is missing. × about 50 diameters.

FIG. 2. A complete specimen slightly older than that of Fig. 1. The first proglottids are forming from behind forwards in typical cestode fashion. × about 50 diameters.

FIG. 3. The posterior region of a specimen in about the stage of Fig. 2, showing a very long terminal proglottid which is bent in two places, but does not show any lines of division. Compressed when killed and hence apparently wider than Fig. 2. × 18 diameters.

FIG. 4. A similar specimen showing a condition which suggests that a long terminal proglottid of an earlier stage has begun to divide into two. × 18 diameters.

FIG. 5. A specimen slightly older than Fig. 2. The scale of 50 diameters being the same as that of Figs. 1 and 2 shows the marked increase in size. × 50 diameters.

FIG. 6. Posterior portion of a specimen somewhat older than Fig. 5. To show typical dimensions of proglottids at this stage. The last division line is faint as though a longer terminal proglottid had just divided. × 18 diameters.

FIG. 7. An entire specimen in which the "anterior proglottids" are appearing. This is actually much larger than the specimen shown in Fig. 5. × 18 diameters.

FIG. 8. The anterior segments of a specimen which still showed an extensive non-segmented zone. × 42 diameters.

FIG. 9. The last six segments from the posterior end of a specimen similar to the one used for Fig. 8. × 42 diameters.

FIG. 10. A specimen of about the same stage as Fig. 7, but with fewer "anterior" and more "posterior" proglottids. A deeper constriction is noticed between the eighth and ninth proglottids from the posterior end. × 18 diameters.

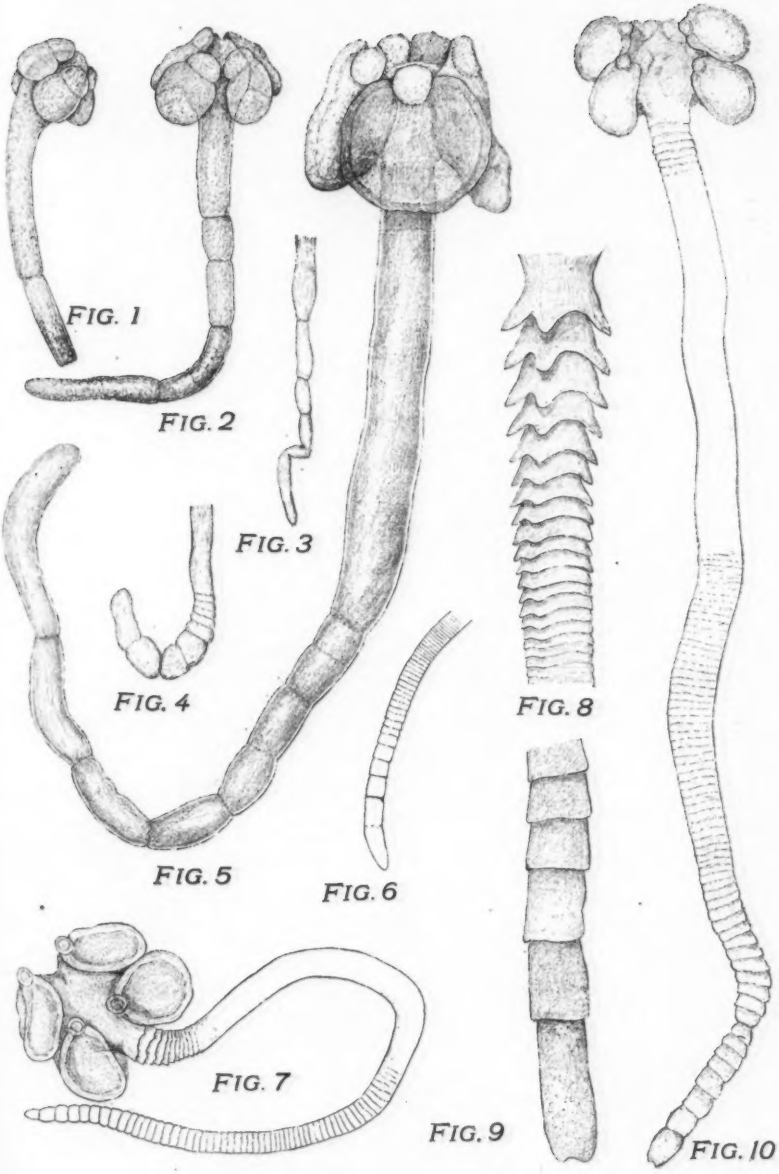

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8

FIG. 9

FIG. 10

PLATE V.

Figures of late stages in the proglottid formation of *C. laciniatum*. All from camera lucida drawings. The magnifications are for the figures as reproduced.

FIG. 11. A specimen with 8 "anterior" and 35 + "posterior" proglottids and showing a greatly elongated, non-segmented zone. × 12 diameters.

FIG. 12. An entire specimen in which the non-segmented zone has been greatly reduced by the appearance of 30 + "anterior" and 200 + "posterior" proglottids. × 12 diameters.

FIG. 13. The non-segmented region of a specimen in which the "anterior" and "posterior" segments are just meeting to obliterate the non-segmented area. Such a stage would be slightly beyond that of Fig. 12. In this instance there were 40 + "anterior," 250 + "posterior" proglottids. × 50 diameters.

FIG. 14, *a–n*, represents the appearance of the proglottids and their proportions in a young adult specimen which contains about its maximum number and is just beginning to liberate ripe proglottids at its posterior end. The length of the regions not drawn in is represented by the straight lines. The letters show the sequence in which the pieces should be arranged. The number of proglottids up to that point is marked at the posterior end of each section drawn. The total number of proglottids was about 506. × 12 diameters.

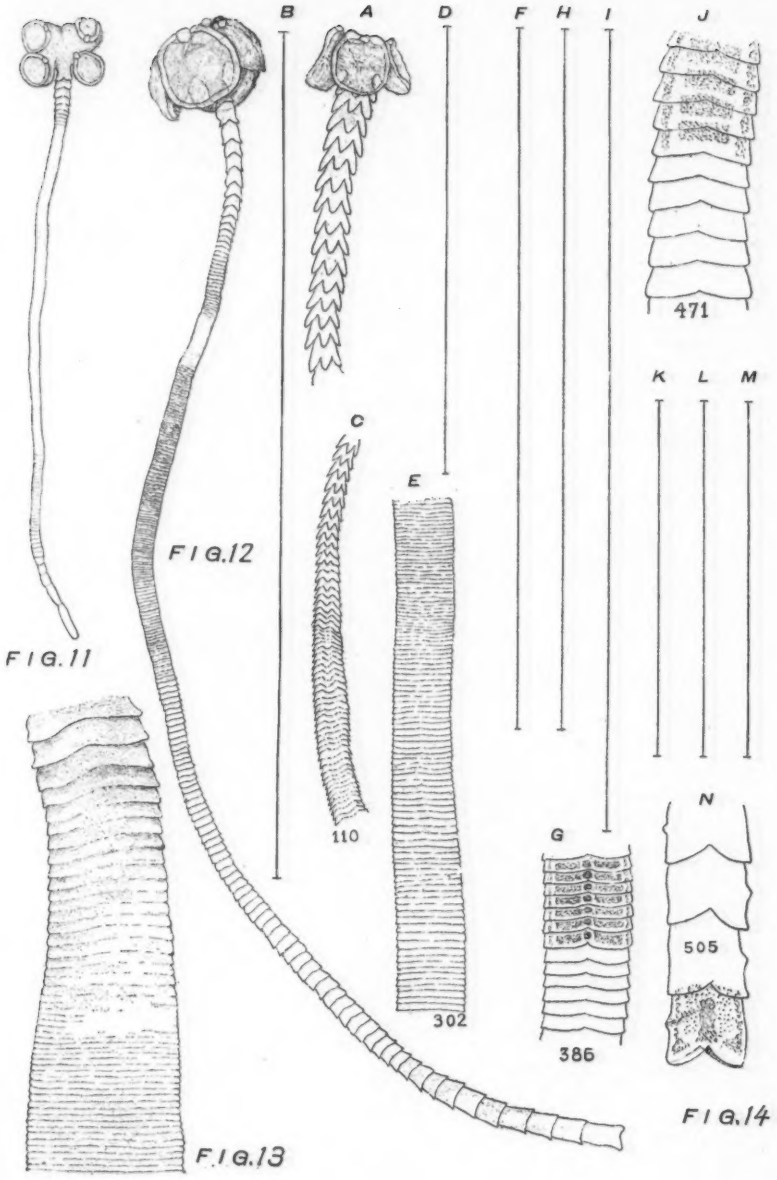
B
A
D
F
H
I
J
471
K
L
M
C
E
110
302
G
386
N
505
FIG. 11
FIG. 12
FIG. 13
FIG. 14

PLATE VI.

Figures showing the development of the "long-necked" specimens of *C. laciniatum*. All from camera lucida outlines. The magnification is given for the figures as reproduced.

FIG. 15. A "long-necked" specimen in which the reproductive organs can be made out as far forward as the fifth proglottid. The tenth and last is about ready to be shed. × 12 diameters.

FIG. 16, *a* and *b*. The entire length of a specimen which is approaching the "long-necked" condition. There is a total of 98 segments and the reproductive organs can be distinguished as far forward as the twenty-sixth proglottid. × 12 diameters.

FIG. 17. A "long-necked" specimen with 8 of the old anterior proglottids and the new segmentation begun in the posterior region of the neck. × 12 diameters.

FIG. 18. A "long-necked" specimen in which the new segmentation has not yet begun. × 12 diameters.

FIG. 19. A "long-necked" specimen with an unsegmented zone further back. See text for a possible explanation of this specimen. × 12 diameters.

FIG. 20. A "long-necked" specimen which has still 49 of the old proglottids in which reproductive organs can be made out as far forward as the third or fourth from the anterior end. The neck region has begun to segment at either end. × 12 diameters.

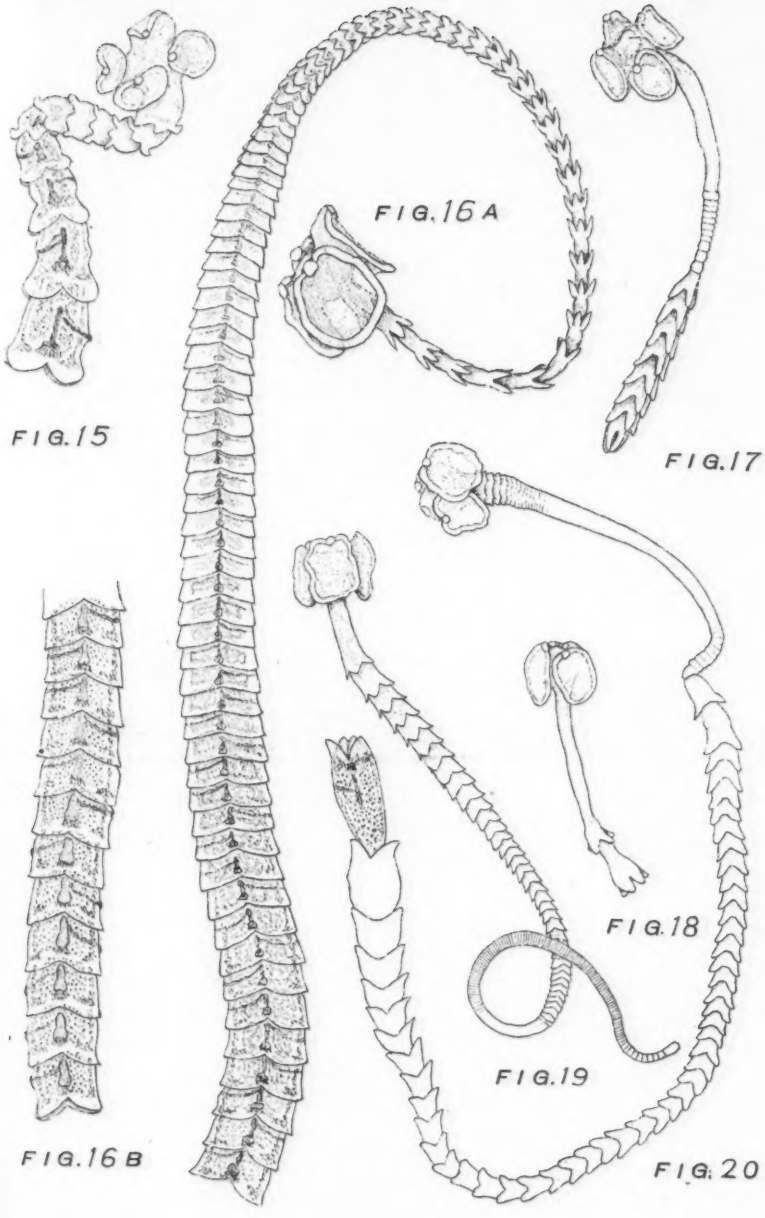

FIG. 15

FIG. 16 A

FIG. 16 B

FIG. 17

FIG. 18

FIG. 19

FIG. 20

PLATE VII.

Figures showing miscellaneous points in the proglottid formation of *C. laciniatum*. All from camera lucida drawings. The magnification is given for the figures as reproduced.

FIG. 21. A specimen which has just entered the "long-necked" condition. There are 34 old proglottids. Reproductive organs probably occur in front of the seventeenth proglottid, but as the specimen was stained for another purpose this cannot be determined with certainty. × 12 diameters.

FIG. 22. A "long-necked" specimen with a single one of the old "anterior" proglottids attached and segmentation beginning in the posterior part of neck region. × 12 diameters.

FIG. 23. Part of a young specimen showing a short non-segmented zone with an abrupt transition of the "anterior" proglottids into the zone. There were in this instance 30 "anterior" and upwards of 217 "posterior" proglottids. × 50 diameters.

FIG. 24. A "long-necked" specimen with a single "anterior" proglottid of the earlier chain still attached at the posterior end and the new segmentation well begun at either end of the neck region. × 12 diameters.

FIG. 25, *a*, *b* and *c*. To show the characteristic shapes of the "anterior" (*a*) and the "posterior" proglottids (*c*). Also the unsegmented zone which separates them. All from the same specimen. There were 35 anterior and 250 + posterior segments. × 50 diameters.

FIG. 26. Part of a transverse section through the non-segmented zone of a specimen about the stage of Fig. 7, Pl. I. See discussion on page 216 of the text.

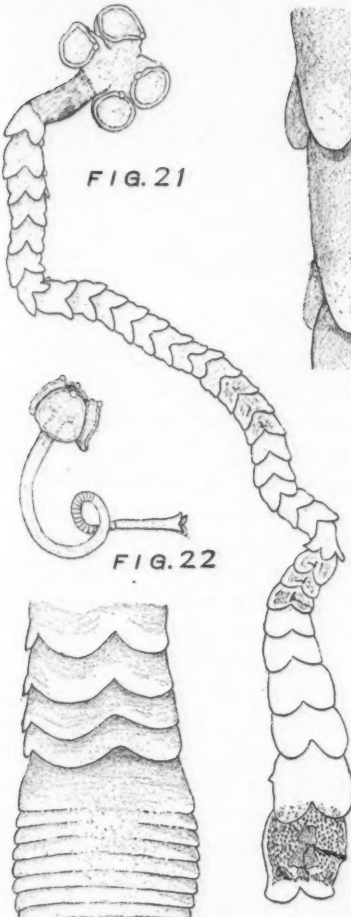

FIG. 21

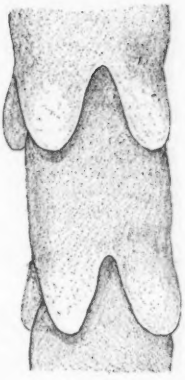

FIG. 25 A

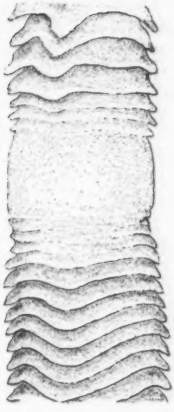

FIG. 25 B

FIG. 22

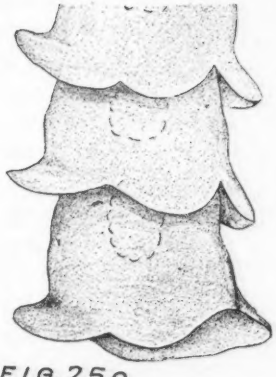

FIG. 25 C

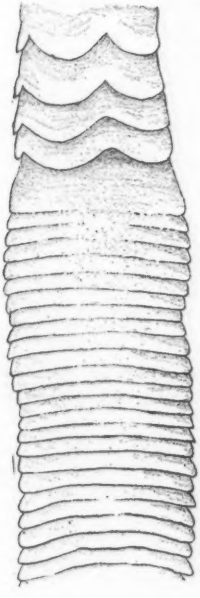

FIG. 23

FIG. 24

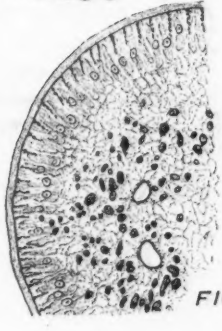

FIG. 26